Cattle
and
Buffalo Farming

HANDBOOK FOR VETERINARIANS

SAREC / NARESA Buffalo Research
and Development Programme
Peradeniya Sri Lanka

Cattle and Buffalo Farming

Handbook for Veterinarians

Edited by

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for the

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Foreword

National Science Foundation (NSF), which is the successor to Natural Resources, Energy and Science Authority of Sri Lanka (NARESA) is proud to be associated with the Water Buffalo Research and Development Programme sponsored by the Swedish Agency for Research Cooperation with Developing Countries (SAREC). Water buffalo and cattle have played a significant role in Sri Lankan society. It was a source of nourishment, farm power and wealth for rural peasants. Over the years this traditional agrarian activity has evolved into a semi-commercialized, organized industry but the industry is now faced with many challenges. With the dawn of the free market economy, the domestic buffalo and cattle industry is compelled to be more productive and efficient. This is a formidable challenge faced not only by the livestock sector but also by the other agricultural sectors. The role of scientific community at this juncture should be to assist the local industries to be more productive, sustainable and more competitive. The scientists who participated in the SAREC/NARESA Water Buffalo Research Programme not only embarked on an ambitious project for generating appropriate and sustainable technologies, but also continued to work until the research information reached the end-users, particularly the rural small farmer.

Documentation of research information and presenting this information in user friendly manner has been one of the many activities pursued under the 3rd Phase of the Water Buffalo Research and Development Programme. This handbook is one of a series of 21 publications put out by this Programme directed towards veterinarians who are playing a key role in providing service functions to smallholder buffalo and cattle farmers. I have no doubt that this publication would become a most valuable manual to veterinarians.

Professor Kapila Dahanayake
Chairman
National Science Foundation

13th December 1999

Preface

This publication presents the most recent information on cattle and buffalo production generated through the dedicated research and extension efforts efforts of Sri Lanka livestock scientists over a period of 20 years. The significant achievements made by the research was made possible by the generous support extended to Sri Lanka by the Swedish Government which sponsored the Water Buffalo Research and Development Programme through the Swedish Agency for Research Corporation with Developing Countries (SAREC).The programme actively supported and administered by the Natural Resources, Energy and Science Authority of Sri Lanka (NARESA, presently the National Science Foundation). The programme which strove to develop new technologies to stimulate buffalo production has borne much fruit. The valuable information which resulted from the long-term research collaboration has been incorporated into a basket of appropriate management practices for the exploitation particularly of the buffalo. Although, useful information generated during the early phases of the programme had been published in proceedings of National and International Symposia, very little of the information had much value to end-users for practical application, as has been the case with research in many other scientific disciplines. Recognizing the deficiencies in the conventional research/extension approaches, the SAREC funded the Buffalo Information Dissemination Programme, as the terminal phase of the SAREC/NARESA Water Buffalo Research and Development Programme, to ensure that the research findings reach the end-users. The objective of terminal phase of the programme was to promote the dissemination of information on the new technologies that had been generated to a wider spectrum of end-users, namely farmers, undergraduates and postgraduate students, extension workers, field veterinarians, livestock specialists, researchers and academics.

As the information generated by the Buffalo R & D Programme is equally relevant to cattle, the SAREC/NARESA Water Buffalo Information Dissemination Programme has included the application of the research information and new technologies to dairy production as well, in the farmer leaflets and the training manual. This Handbook for Veterinarians is the next in the series of extension publications designed to cater to the needs of the different target groups.

Based on the experiences gained and the information gained from the long term interactions with field veterinarians and farmers through continuing education programme, we are realised that there is a urgent need for a field manual on dairy and buffalo management for Veterinarians to serve as reference manual. This publication is the result of our committment to fulfil this long felt need to disseminate the vast amount of information that has been generated over the last 15 years.

The Handbook is arranged in six parts, (1) feeds and feeding (2) reproduction and obstetrics (3) breeding (4) health (5) pharmacology and therapeutics and (6) management. For the convenience of the reader, a subject index has been included for quick reference.

We wish to express our sincere appreciation to the former Director General of NARESA, Prof. Priyani Soysa for the constant encouragement and constructive criticism and the Chairman of the National Science Foundation Prof. Kapila Dahanayake for the assistance his assistance in

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various ways. Our thanks are due to Mr. Wasantha Amaradasa, Deputy Director (Scientific Affairs) for the excellent cooperation extended to us, and to the authors of the various chapters, who have worked hard to present the information to meet the needs of the Veterinarians in a readable manner. We also wish to express our sincere thanks to Mr. Janaka Herath and Mrs. Ayesha Senarath Bandara for assisting in the computer work and Mr. D.K. Niroshan for assisting in the art work.

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CHAPTER 1

Ruminant Management Systems, Feed Resources and Constraints to Feeding of Ruminants

J. A. de S. Siriwardene

Introduction

Forage constitutes the cheapest form of feed for ruminants. The traditional feeding systems practised in Sri Lanka are based on the forage resource base within each of the production systems, which in turn is largely influenced by climatic and environmental variations during the different seasons of the year. The major traditional feeding systems fall into five broad groups (Fig. 1.1) and combination of these systems. Year round feed availability is affected by factors such as the rainfall pattern, cropping systems and intensity of production.

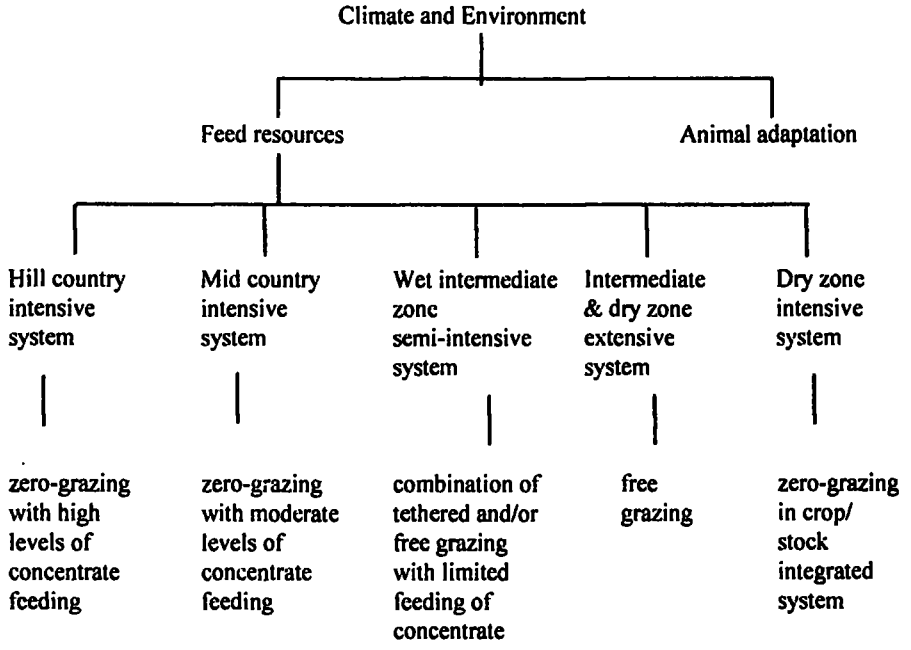

Fig. 1.1 Major feeding management systems

Feed availability, which is dependent on the level of precipitation, is a function of the balance between the rate of evaporation, the rate of precipitation and the length of the dry periods. Under extensive management systems, ruminants obtain most of their nutrients from grazing or browsing. During the wet season, when abundant green forage feed is available, animals obtain adequate good quality pasture through selective grazing. However, during the dry periods, the availability and quality of forage declines, limiting intake and selectivity during grazing. Spells of dry weather that occur between periods of rain are experienced in almost all parts of the country, leading to a decline in the quantity and quality of forage. This is evident even in the

hill country during January and February when dry weather combined with frost, depletes the forage resources in open fields.

It is therefore important to recognise the need to develop feeding systems for each agro-climatic zone, based on the feed resource base and quality, and the year round availability of the feed resources. Under tropical conditions, plant growth slows down and often ceases during dry spells, and pasture and fodder mature rapidly and decline in quality. Problems associated with the capability of the farmer to provide adequate feed of good quality are encountered generally during the dry periods.

Farmers in Sri Lanka are basically crop farmers. It has been estimated that only 30 % of crop farmers rear some type of livestock. The difficulties in finding time and labour particularly during the cultivation and harvesting seasons, prevent farmers from engaging in dairy activities in mixed farming systems. This is also the reason why dairy farmers have not resorted to forage conservation as a means to overcome feed shortages during dry periods, a procedure that is widely practised in countries such as India and Pakistan. Scientists have so far not been able to come up with a practical and appropriate procedure to help livestock farmers to overcome the continuing problem of feed scarcity during the dry seasons of the year. The most compelling task facing planners, researchers and extension workers associated with cattle and buffalo production is therefore to do with the development and utilisation of feed resources. These resources can be grouped into (1) pasture and fodder crops (2) fibrous crop residues (FCR) (3) agro-industrial by-products (AIBP) and (4) non-conventional feed resources (NCFR).

Chapters 3 to 6 of this Handbook which deal with practical aspects of ruminant nutrition will provide an understanding of ways and means of utilising the available feed sources more effectively, combined with manipulation of the rumen environment, by strategic use of feed supplements to improve the efficiency of feed utilisation. In order to achieve this objective, it is essential for the reader to understand the basic principles of ruminant nutrition and formulation of balanced rations for dairy animals (Chapter 2 and 6).

This introductory chapter will deal with the feed resources available to small holder farmers and provides some information on the seasonal availability and quality of feed resources.

1.1 Traditional grass and fodder resources

The major traditional sources of feed for ruminants are pasture and fodder, which includes tree fodder. Ruminant production in tropical countries is based to a very large extent on nutrients derived from grazing on natural pasture. Attempts made during the last two decades to establish improved grass and fodder varieties through a pasture subsidy incentive scheme, have not had a significant impact. It is estimated that the extent of improved pasture is no more than 5 percent of the total area under pasture. The important species of grass and fodder that support the dairy industry are, Guinea grass in the mid country, dry zone and parts of the intermediate zone; *Brachiaria spp.* and natural pasture varieties in the coconut triangle and parts of the dry zone; and a

combination of Guinea grass and a large number of natural pasture species in the rest of the country. The pasture production curve follows the rainfall pattern during the year. Fig. 1.2 shows the typical pasture production curve in regions where precipitation is highest during the Maha monsoon season. The pasture production curve that applies to other areas too follow a similar bimodal pattern but may vary depending on the level of precipitation in these regions.

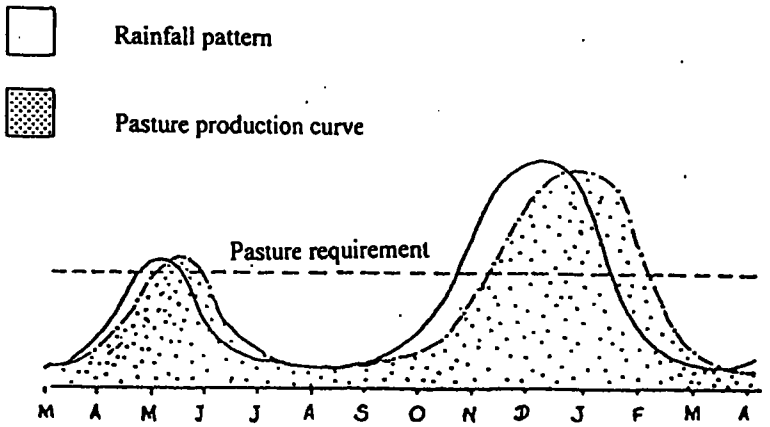

Fig. 1.2 Annual pasture production curve

Basically, the problems encountered by farmers in feeding their stock and maintaining production levels, emerge during periods of low pasture productivity. It is to meet such contingencies that pasture conservation is practised in most countries. However, these practices have not found acceptance by local farmers. Farmers instead resort to feeding of concentrates which is not cost effective.

Table 1.1 Annual yields of pasture and fodder (kg DM/ha/yr.)

Pasture species	Jothiland Estate[1]	Haragama Farm[2]	VRI Gannoruwa[3]	TRI Hantana[4]	CRI Luñuwila[5]
P. max. (Guinea A)	7,500	14,527	35,438	-	-
P. max. (Guinea B)	5,713	13,273	28,037	-	8,379
P. max. cv. Guinea	-	14,367	34,448	24,815	-
P. max. cv. Hamil	8,520	18,713	-	-	-
B. brizantha	8,473	13,273	-	-	3,975
B. ruziziensis	6,747	12,587	-	23,920	3,990
B. milliformis	-	-	-	-	2,518
B. decumbens	7,840	15,707	-	16,260	

Source: 1. and 2. Chadokar, P. (1982)
3. Pathirana, K. K. and Siriwardene (1973)
4. Pathirana, K.K. and Siriwardene, J. A. de S. (1980)
5. CRI Annual Report (1983)

Research results show a wide variation in the yield figures of pasture and fodder

varieties grown under experimental and field conditions. This is an indication of the effect of the variation in soil types and climatic conditions in different locations. For instance, at Jothiland Estate in the mid-country, the average yields were between 5,500 to 8,500 kg DM/ha/year with pure grass swards fertilised at the rate of 100 kg N/ha/year. However at Haragama, yields of between 12,000 to 18,000 kg DM/ha/year have been recorded in the first year of cultivation, but declined in later years due to depletion of soil organic matter and low rainfall. The highest yields recorded in the mid-country region, at the VRI Peradeniya on good soil, ranged from 26,280 kg DM/ha/year with *S. sphacelata* to 35,438 kg DM/ha/year with Pusa Giant Napier. The yields obtained with similar varieties cultivated on marginal tea land at the TRI Mid-country Station at Hantana were relatively low, while those on coconut land were surprisingly low (Table 1.1). It is obvious that although improved grasses have high production potential under of good management, they perform no better than the local ecotype of Panicum maximum (Guinea A) under relative poor management conditions that are obtainable in small farms.

Table 1.2 gives the composition of Guinea grass at different stages of growth. It is evident that as grasses mature, the crude protein content declines and the level of crude fibre and cell wall contents increase, resulting in a drastic reduction in the essential nutrient contents and digestibility. Grass and fodder should therefore be grazed or harvested at the stage of growth that will provide the highest nutrient levels. It is evident that guinea grass harvested at three week intervals can provide relatively high levels of nutrients to dairy animals.

Table 1.2 Composition of Guinea A grass at different stages of growth

Description	DM	Ash	CF	CP	NDF	ADF	Lignin
Panicum maximum (Guinea A)							
1 week harvest	16.3	-	-	14.1	69.9	41.9	65
3 week "	23.5	11.8	33.3	12.6	68.3	38.4	53
4-5 week "	28.0	11.2	37.4	10.2	72.7	44.6	56
8-9 week "	28.2	11.7	44.4	5.8	74.8	50.3	73
12 weeks "	30.1	7.7	46.2	3.1	77.4	53.6	79

Source: Ibrahim, M. N. M. (1988)

1.2 Potential feed resources

The Statistical Abstracts (1996) of the Department of Census and Statistics of the Ministry of Finance and Planning gives the estimated populations in 1995, of cattle, buffaloes and goats as 1.9 million, 800,000 and 500,000, respectively.

The pasture land that is available is categorised into four groups (Table 1.3) on the basis of the assumed annual pasture production potential of each category in terms of dry matter. For example, it is assumed that unirrigated highlands in the dry zone produce one MT of pasture, while land under coconut yields 4 MT of pasture in terms of DM per year. The extent of pasture land and the total production of grass and

fodder at the present time is estimated at 707,500 ha and 1,716,500 MT of DM per annum respectively. This resource cannot support the feed requirements of the cattle, buffalo and goat populations in the country. While the deficit in fodder requirement in relation to fodder production has been estimated to be 2.8 million MT, it has been shown that this deficit could be balanced with the use of the crop residues that remain are largely unutilised. Even if these estimates are taken as rough indicators of the extent of the fodder resource availability, it is clear that the grass and fodder resources alone are inadequate to meet the feed requirements of ruminants. Supplementation of grass and fodder with crop residues and agro-industrial by-products is the only means available to bridge the deficit in the feed supply (Table 1.4). The potential availability of crop residues is estimated to be 3,254,526 MT per year and that of non-conventional feed resources was estimated at 230,291 MT in 1984 (see Table 3.4 in Chapter 3).

The challenge for the future is to develop technologies that will make it possible to use the resources available, in a form that can be utilised for direct feeding or for incorporation in commercial production of feed mixtures.

Table 1.3 Extent of land under pasture and the estimated yield of pasture

Categories of pasture land	Extent of land (in ha) based on annual dry matter yield of pasture (kg/ha/yr.)				Total DM production (MT/yr.)
	1MT DM/yr.	2MT DM/yr.	4MT DM/yr.	7MT DM/yr.	
Unirrigated highlands in dry zone	325,000	-		-	325,000
Villus in dry zone		-	50,000	-	200,000
30% of land area under coconut	-	-	140,000	-	560,000
5% of hill country tea estate land	-	-		4,500	31,500
Patana land in hill country	55,000	-	-	-	55,000
Herbage from paddy lands (2 months of grazing between seasons)	120,000	-	-	-	120,000
Road sides etc.	-	5,000	-	-	10,000
Grazing land in wet zone	-	20,000	-	-	40,000
Fallow paddy lands	-	150,000	-	-	300,000
Total	500,000	190,000	-	4,500	1,586,500

Plus the extent of improved pasture - 13,000 ha x 10 MT/yr.	= 130,000
Total pasture production (MT DM per year)	= 1,716,500

Source: Revised estimates based on Piyasinghe, W. (1984)

Scientists have been working towards finding solutions to these issues. Technologies that were developed earlier, to utilise tree fodder, fibrous crop residues, (such as rice straw) and agro-industrial by-products (such as rubber seed meal, tea refuse, bagasse and sugar cane tops) have not been adopted at both industrial and farm level,

because of problems associated with processing and utilisation. In the past farmers faced difficulties in adopting these procedures in practical feeding. A good example was the failure of the research and extension efforts in the past to promote the utilisation a urea-treated straw by farmers. Despite the successes achieved by scientists in improving the feeding value of rice straw and demonstrating the value of urea treated straw as a supplementary feed through on-station and on-farm trials, farmers were not willing to adopt this technology for the simple reason that these technologies were inappropriate at farm level.

Learning from past experience, scientists working with the SAREC/NARESA Buffalo Research and Development Programme have been successful in developing appropriate technologies through the application of the farming system research approach. In this system, the farmer is an active player in the development and refinement of technologies at all levels in the research and development effort.

Table 1.4 Availability of fibrous crop residues

Crop residue	Extent of land cultivated (ha)[1]	Total yield of primary crop (MT/yr.)	Grain (or crop) to residue ratio	DM yield of crop residues (MT/yr.)
Rice straw	863,439	2,810,000	1:1	2,810,000
Maize stover	35,938	34,800	1:2[2]	71,876
Kurakkan straw	6,339	4,900	1:3[2]	14,700
Meneri straw	340	200	1:3[2]	600
Sorghum stover	241	220	1:3[2]	660
Green gram	18,097	16,100	1:1	16,100
Cowpea residue	18,105	16,100	1:1	16,100
Dhal	30	-	1:1	30
Gingelly	8,560	4,500	1:1	4,500
Ground nut	9,896	-	1:5[2]	7,710
Manioc leaves and stem	32,824	288,700	2:1	144,350
Sweet potato vines	9,125	61,800	2:1	30,900
Sugarcane tops		685,000	5:1[3]	137,000
Total				3,254,526

Sources: 1. Statistical Abstracts (1996)
2. Joshi, A.L. *et al.*(1988)
3. Preston, T.R. (1995)

The information provided in Chapters 3 to 6 includes the most recent developments in technology relating to nutrition, which have now been incorporated into management packages. The reader should appreciate that the feeding recommendations given in the following chapters do not exclude the application of the traditional feeding management systems. These are in effect are intended to improve the traditional feeding systems through utilisation of the potential feed resources and supplementation of deficient nutrients. The objective of these recommendations are primarily to (1) bridge the gap between feed requirements and feed availability (2) balance the nutrient deficiencies so as to improve these nutritive value of the diet and

(3) manipulate the rumen environment to ensure optimum microbial growth and activity. These three approaches combined together make it possible to utilise hitherto unutilised or under-utilised feed resources, to increase the productivity of dairy animals.

It should be clearly understood that there still remains an urgent need to improve the basal forage diet by cultivation of improved high yielding grass and fodder varieties. Farmers should realise that increasing the quality of the feed resource base is a necessary prerequisite to the rearing of genetically superior dairy animals.

Selected references

Annual Report (1988) Coconut Research Institute, Lunuwila.

Chadokar, P.A. (1982) *Recommendations for tropical forage development.* UNDP/FAO Tropical pasture development project. SL/IDA Dairy Development Project, Getambe, Peradeniya.

Ibrahim, M. N. M. (1988) *Feeding tables for ruminants in Sri Lanka.* FFUP Publication.

Joshi, A. L, Prabhu, U.H. and Sampath, S.R. (1998) Treatment and supplementation of finger millet (*Eleusine* coracana) and sorghum (*Sorghum bicolor*) straw. In: Fibrous *crop residues as animal feed.* ICAR. New Delhi, India, pp. 17-23.

Pathirana, K. K. and Siriwardene, J. A. de S. (1973) *Ceylon Veterinary Journal.* **21:** 52.

Pathirana, K. K., Nugara, N.M. St. D., Siriwardene, J.A. de S. and Jayawardene, A.B.P. (1980) *Ceylon Veterinary Journal* **28:**19-23.

Piyasena, M.A. (1984) *A study of animal feed resources in Sri Lanka.* Ministry of Rural Industrial Development, Colombo.

Preston, T.R. (1995) *Tropical animal feeding.* FAO Animal Production and Health Paper 126, p 150.

Preston, T.R. and Leng, R.A. (1987) *Matching ruminant production systems with available resources in the tropics and sub-tropics.* Penambul Books Ltd., Armidale, Australia.

Siriwardene, J. A. de S. (1985) Relevance of crop residues and non-conventional feeds in overcoming feed shortages. In: *Potential of rice straw in ruminant feeding - Research and field application.* Straw Utilisation Project. Publ. No. 1. pp. 35 - 54.

Shaw, J.R., Capper, B.S. and Manser, P.J. (1979) *A strategy for the development of livestock feed industry in Sri Lanka.* Tropical Products Institute, London. UK.

Statistical Abstracts (1996) Department of Census and Statistics.

CHAPTER 2

Principles of Ruminant Nutrition

M. N. M. Ibrahim, H. Abeygunawardena and J. A. de S. Siriwardene

Introduction

Ruminant animals (buffaloes and cattle) possess a complex stomach made up of four compartments - the rumen, reticulum and omasum, which constitute the fore-stomachs and the abomasum, the true stomach. This complex stomach gives ruminants the added advantage in its ability to utilise fibrous feeds and convert these into food products - milk and meat. For this reason, most of the nutrient requirements of ruminants could be provided through roughage or fibrous feed.

A schematic diagram of the ruminant digestive system is given in Fig. 2.1

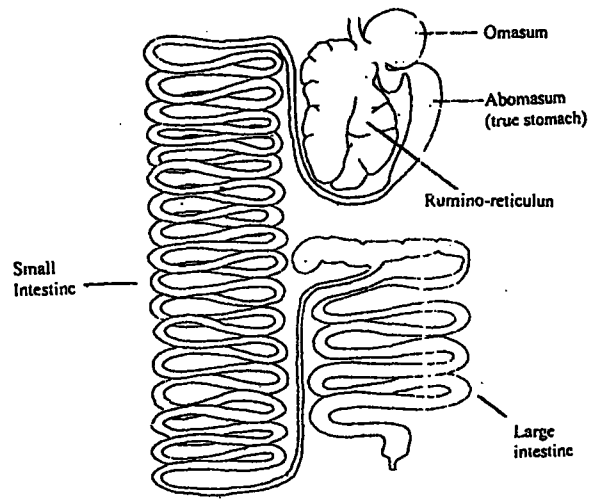

Fig 2.1 Schematic diagram of ruminant digestive system

The digestion of fibrous feeds begins in the sac-like compartments where the breakdown process occurs through microbial fermentation. The microbes in the rumen are normal inhabitants of the complex stomach. Digestion commences in rumen and the feed passes through reticulum and omasum ending in the abomasum and thereafter continues into the small intestine. The digestion in the reticulo-rumen occurs through the action of microbial enzymes and not through digestive enzymes as in digestion in the small intestine. For this reason, ruminants are able to use fibrous feeds which are not digestible in simple stomached animals such as the horse, pig and dog. The advantage of the ruminant stomach is its ability to (1) digest cellulose, hemicellulose and pectins which are cell wall constituents (2) utilise non-protein nitrogen (NPN) and

obtain protein through microbial protein synthesis in the rumen and (3) utilise dietary lipids more efficiently for productive purposes.

2.1 Rumen microbial digestion

Basically the rumen has three functions in relations to digestion.

(1) Fibre digestion
(2) Microbial protein synthesis
(3) Vitamin B synthesis

These functions are carried out through rumen microbial activity. About 80-90 % the roughage fibre is fermented and the total protein requirements for maintenance is obtained through microbial activity. The efficiency of rumen function is therefore influenced by the level of microbial activity, which is determined by the density of the microbial population and the nutrient supply to the microbes. The vigour with which microbes break down food entering the rumen, depends upon rate at which they grow and reproduce. In order to grow and reproduce, microbes must have sufficient energy, nitrogen (protein) and minerals to meet their requirements. Furthermore, the ionic content as reflected by pH in the rumen too is an important factor. The ruminant diet therefore has to provide an optimum rumen environment where an optimum pH and a balanced supply of nutrients is available in the rumen liquor to support and promote microbial growth and multiplication. The type and composition of the diet consumed by the animal plays a vital role in overall nutrient utilisation by the animal.

A simplified schematic diagram of digestive utilisation of feed by ruminants is given in Fig. 2.2.

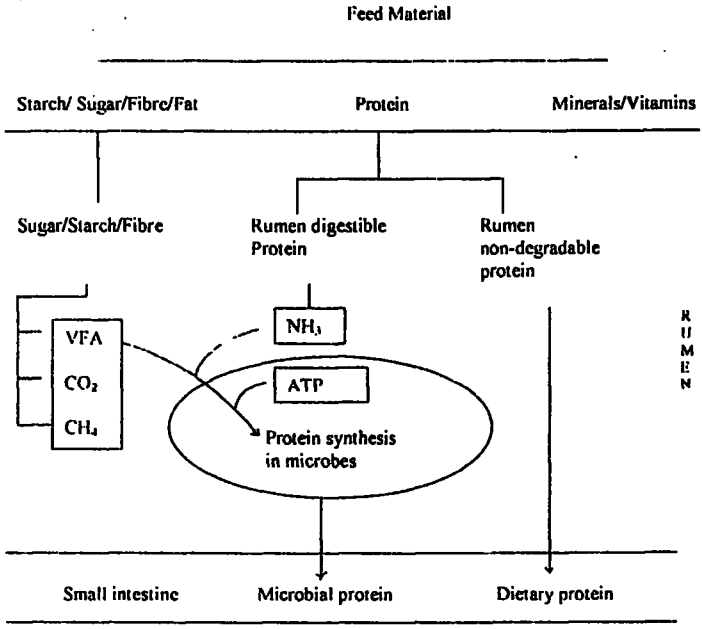

Fig 2.2 Utilisation of rumen breakdown products in bacterial protein synthesis

The feed that enters the rumen is subjected to anaerobic fermentation through the activity of the dense microbial population consisting of bacteria, protozoa and fungi. The breakdown products are volatile fatty acids (VFA), rumen ammonia (NH_3), short carbon chains, methane and minerals. About 60 percent of the digestible energy of the feed is derived from VFA. VFAs are absorbed from the rumen by diffusion across the rumen wall while about 25 percent is absorbed post-ruminally. A proportion of the VFAs, ammonia and minerals are utilised by microbes as nutrients for their growth and multiplication. When these microbes eventually pass down into the intestine, the microbial protein is digested by the proteolytic enzymes with the release amino acids. The rumen non-degradable proteins too pass into the small intestine and are digested along with microbial protein. Thus the animal receives protein from two sources – dietary protein (non-degradable or by-pass protein) and microbial protein, where the latter source contributes the major share. The absorption of the final breakdown products occur through the wall of the complex stomach as well as through the small and large intestines.

2.2 Carbohydrate fermentation

Dietary carbohydrates are present in the rumen in two forms, as (a) cellulolytic or structural carbohydrates (insoluble form) and (b) starch and sugars (soluble form). The major activity of rumen microbes is the digestion of the structural carbohydrates. However, in order to digest the structural carbohydrates, microbes need readily available energy, which is available from soluble carbohydrates found in plant cell contents. Energy from this source is available for utilisation immediately after ingestion of the feed. Thus, diets high in soluble carbohydrates enhance microbial growth and multiplication, improving the efficiency of utilisation of feed. This readily available energy permits microbes to use the ammonia and amino acids derived for the diet and also the ammonia recycled through saliva, for rapid growth. Once the soluble carbohydrates are utilised, the microbes have to depend on the structural carbohydrates (cellulose, hemicellulose and pectins associated with lignin) as the energy sources. There is a time lag between ingestion of feed and fermentation of structural carbohydrates as the rate of fermentation of fibre is slow. It is during this period that the demand for ammonia and energy will exceed the rate of supply. Therefore the greatest benefit from supplementation of energy is obtained during this period.

However, the incorporation of excessive levels of soluble carbohydrates in the diet must be avoided as this can lead to rapid acid production in the rumen, which can cause metabolic disturbances such as acidosis. Where NPN is used as a source of nitrogen, relatively higher levels of soluble carbohydrates must be provided in the diet. The soluble carbohydrates sources generally used in ruminant diets are rice bran, rice polish, maize and molasses. Compared with other soluble carbohydrate sources, molasses contain higher levels of easily fermentable sugars and micro mineral elements, which are essential to the ruminant. Thus molasses can be considered as the most effective and economical soluble carbohydrate source for ruminants. Molasses can be incorporated in the feed up to a level of 10 –15% on DM basis. The other advantage in using molasses is that it improves the palatability, inducing a higher feed intake. A low pH in the rumen will suppress the activity of cellulose digesting bacteria

and cellulolysis, resulting in low fibre digestion and utilisation in the rumen. Therefore, a continuous supply of appropriate levels of soluble carbohydrates in the diets must be ensured. Agro-industrial by-products, immature grass and tree fodder are also good sources of soluble carbohydrates. When fed with coarse roughage, they provide substantial amounts of soluble carbohydrates, which supplement the deficiencies in poor quality feeds.

For optimum digestion, microbes require a rumen environment containing a balanced supply of nutrients (ammonia, VFA, soluble sugars and minerals) and an optimum pH of 6.9. Of the factors influencing fermentation in the rumen, ammonia concentration plays a critical role. The effect of rumen ammonia concentration on intake and digestibility of fibrous feed are depicted in Fig. 2.3. Thus the optimum concentration of rumen ammonia nitrogen for optimum utilisation of low quality forage is in the range of 180 to 200 mg per litre of rumen fluid.

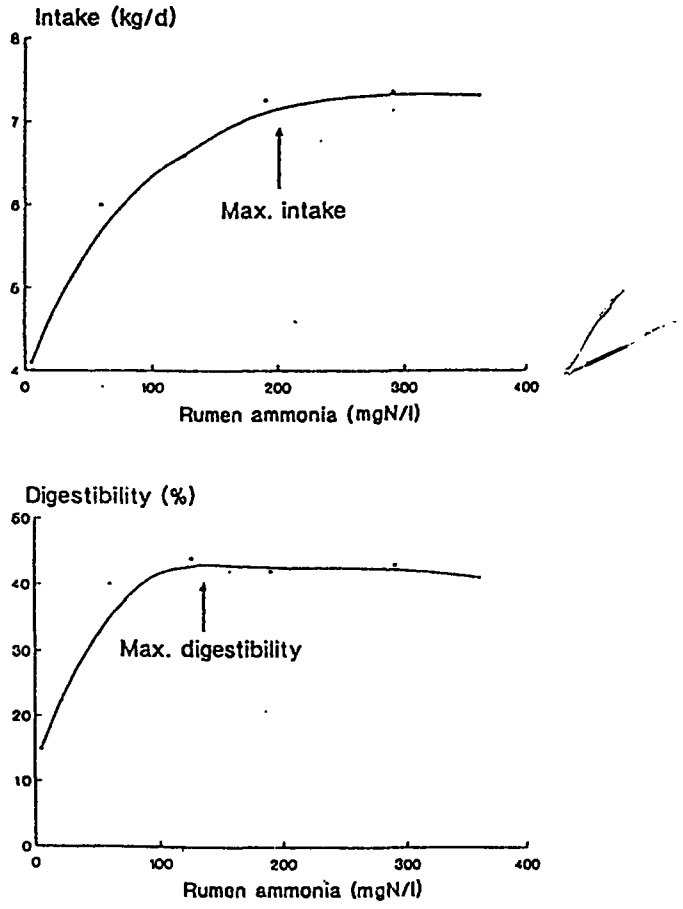

Fig. 2.3 Effect of rumen ammonia concentration on feed intake and digestibility. (Perdok, H.B., 1987)

2.4 Nitrogen metabolism in the rumen

The main sources of nitrogen for rumen microbes are dietary protein and non-protein nitrogen (NPN). Most of the protein that enter the rumen are degraded to peptides and amino acids, which are mostly deaminated releasing ammonia and branched-chain VFA. The rate of degradation of protein depends on the rumen environment. Protein in some feeds are more resistant to degradation than in others. The ammonia released contributes to the ammonia pool in the rumen. This ammonia is either incorporated into microbial cells, or absorbed through the rumen wall or to a lesser extent passes out of the rumen in the rumen fluid. The ammonia pool is relatively small but the turnover is rapid. Urea recycled to the rumen from plasma urea forms a significant source of nitrogen to the ammonia pool. The latter source cushions the severity of periodic nitrogen deficiencies during periods between intake of feed.

2.5 Macro and trace elements

The animal body contains about 3% minerals which are normal constituents of animal tissues. In the animal body, there are about 30-40 mineral elements which occur in various parts of the body. Calcium and phosphorus account for three-fourths of the mineral elements in the body. Minerals are required in smaller quantities compared to nutrients like energy and protein. Mineral deficiencies can have a marked effect on productivity, particularly on reproductive performance and health. Mineral requirements are related to animal output, and therefore the use of mineral supplements is particularly important for high producing animals. Mineral elements are classified as macro (Ca, Cl, K, Mg, Na, P and S), micro (Cu, I, Fe, Mn and Zn) and trace elements (Mo, Co, Cr, Ni, Se and V) depending on the requirements of the animal for maintenance of both the content in animal tissues and the biological functions at an optimum level.

Minerals are an important nutritional component of the diet. They are involved in biochemical processes associated with generation of energy. Major sources of minerals for livestock are feed and drinking water. Most of the cell-bound minerals are released in the rumen during the fermentative microbial digestion process. These minerals are readily soluble and are immediately available to the animal as well as to the rumen microbes.

Minerals also play a major role in the rumen in maintaining the buffering capacity, which facilitates the maintenance of the rumen pH at an optimum level for rumen activities. Dietary manipulations are carried out by the addition of sodium bicarbonate or sodium chloride to improve the buffering capacity and nutrient utilisation. Ruminants cannot synthesise the sulphur amino acids, methionine and cysteine from sulphate. The animal relies on the rumen microbes to convert sulphate to methionine and cysteine, necessary for microbial growth.

2.6 Nutritive value of feeds

Nutritive value is a function of the feed intake and the efficiency of extraction of nutrients from the feed during digestion, which in other words, is the digestibility.

Feeds of high nutritive value produce high levels of production. Feed intake in ruminants is determined by the extent and speed of degradation of cell wall components, the release of digestible elements into the rumen fluid and the rate of passage of the digested feed from the rumen. Intake is affected by the palatability of the feed, which has a direct bearing on the physical characteristics (coarseness). It is also influenced by the presence of certain compounds that affect taste. Digestibility of the feed and intake may not be the best indicator of the nutritive value, as this does not give a true assessment of efficiency of utilization of nutrients in feeds. The reason being that this is a function of the balance of nutrients in rumen fluids and the rumen environment.

2.7 Factors affecting feed intake

There are many factors that affect feed intake. Smell resulting from contamination of grass by dung or low palatability due to mould growth may cause animals to reject feed without tasting. Dusty feeds can cause irritation of the nose and eyes and decrease the intake of feed. Free grazing animals tend to be selective and show a preference for feeds with more digestible components. The feed intake is often determined by the rate of absorption of the soluble components of the feed and the rate of passage out of the rumen. The composition of the diet therefore has an influence on the voluntary feed intake (VFI). VFI is reduced by nutrient imbalances in feed, when one or more of the nutrients are limiting. Often the limiting nutrient is ammonia, particularly in low quality diets. The digestibility may not be the most important constraint, as is evident from the response in animal performance when the limiting nutrients are supplied through supplementary feeds. Most often intake is influenced more by nutrient imbalances in feed rather than by low digestibility.

2.8 Manipulation of the rumen ecosystem

The key to understanding feeding of ruminants involves the fermentation of feed and the use of the end products of fermentation. The balance of end-products in relation to the requirements of the animal affects the efficiency of utilisation. Manipulation of rumen fermentation is directed towards achieving three major objectives.

1. To increase the digestibility of structural carbohydrates in the rumen
2. To increase the ratio of propionic acid to the total VFA concentration in the rumen.
3. To change the protein to energy ratio of the end-products of fermentation.

Coarse roughage is not easily fermented in the rumen. However, physical and chemical treatment can be used to increase the rate and extent of degradation. These procedures will be discussed in detail in Chapter 3.

Any deficiencies in the nutrient balance in the rumen environment will reduce microbial activity and result in a reduction in digestibility of the feed. Thus, the primary objective of rumen manipulation is to provide the optimum balance of essential nutrients required for microbial growth. When low quality fibrous feeds are consumed by animals the most important factor that limits microbial growth is a decline in the rumen ammonia concentration. The need to manipulate the rumen

environment so as to ensure the maintenance of the optimum level of rumen ammonia was discussed in section 2.4 above. A number of ways of ensuring the maintaining the optimum ammonia concentration are available. Urea or other NPN sources can be fed with low quality roughage. However, the utilisation of urea fed in this manner is inefficient, as urea is rapidly converted to ammonia causing the concentration to reach a peak within half an hour, thereafter to rapidly decline to below critical levels, until the next intake of feed. Fibre digestion reaches a peak 5 to 6 hours after ingestion. Therefore, feeding urea once a day is ineffective particularly with grazing animals that consume feed evenly throughout the day. Feeding the same quantity of urea many times during the day is much more effective in maintaining the ammonia concentration at steady level than feeding once or twice a day.

Feeding of urea treated roughage is therefore more effective than feeding urea sprinkled straw. Even more effective is the provision of urea through a urea-molasses-mineral supplement in the form of a block, to which the animal has access throughout the day. An alternative method is to provide dietary nitrogen by feeding nitrogen-rich feed supplements such as legumes and leguminous tree fodder.

2.9 Development of feeding systems

The practical application of the principles of ruminant nutrition basically hinges on a clear understanding of the dynamics of rumen digestive processes. It involves the provision of the nutritional and other inputs required to maintain a conducive rumen environment. In practical terms, ration formulation thus requires a knowledge of the daily nutrient requirements for the appropriate physiological status of the animal and the ability to make an accurate assessment of the nutrient content of the daily ration provided to the animal. The reader will now be in a position to appreciate that there is a wide variation in the nutrient composition of feeds and the need to provide through supplementary feeds, the deficient nutrients as a means of improving the efficiency of utilization. Manipulation of the rumen environment through provision of inputs to maintain optimum conditions for microbial growth and activity is an essential component of the feeding management system. Attention to these factors will ensure improved efficiency of feed utilization and animal performance. The subsequent chapters will provide more information on assessment of feeding value and the limitations in the use of feed resources and procedures to overcome these limitations. Utilization of this knowledge will help the reader to formulate appropriate feeding systems utilizing the feed resources that are available in a given management and production system.

Selected references

Banerjee, G.C. (1988) *A textbook of animal husbandry.* (5th Edition) Oxford and IBH Publishing Co., New Delhi, India.

Chesterworth, J. (1992) *Ruminant nutrition.* CTA, Wageningen, The Netherlands.

Church, D.C. and Pand, W.G. (1988) *Basix animal nutrition and feeding.* (3rd Edition) John Wiley & Sons, UK.

Forbes, J. M. (1995) *Voluntary food intake and diet selection in farm animals.* CAB International, UK.

McDonald, G., Edwards, R.A. and Greenhalgh, J.F.D. (1988) *Animal Nutrition* (4th Edition) Longman House, Burnt Hill, Essex, UK.

Preston, G.C. (1995) *Tropical animal feeding.* FAO Animal Production and Health Paper 126. Rome, Italy.

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CHAPTER 3

Utilisation of Tree Fodder and Non-conventional Feed Resources

A. N. F. Perera, J. A. de S. Siriwardene and S. Premaratne

Introduction

As was mentioned in Chapter 1, the objective of the chapters on nutrition is to present the most recent research information on procedures for the utilising potential feed resources available for ruminant feeding. A comment that is often made by foreign visitors to Sri Lanka, is that Sri Lanka has abundance of green vegetation. When compared to the forage resources available in the neighbouring countries like India and Pakistan, Sri Lankan dairy farmers should have no problem in providing feed to sustain a flourishing dairy industry. However, the reverse is true. In spite of the severe shortage of green forage, India is self-sufficient in milk and milk products and is now an exporter of milk. India has achieved this status through the extensive utilisation of non-conventional feeds and agro-industrial by-products.

Sri Lanka has an abundance of forage and other fibrous material, which is either not utilised or grossly under-utilised. The other feed resources besides pasture, fall into three categories (1) tree fodder (2) fibrous crop residues and (3) agro-industrial by-products.

Non-conventional feed resources (NCFR) have been included along with fibrous crop residues (FCR) and agro-industrial by-products (AIBP), for the reason that there is considerable overlap between these categories of feedstuffs, depending on the feeding systems in different countries. Generally, NCFR are either not utilised or marginally used in traditional feeding systems. Examples of NCFR are by-products from the oil extraction industry (other than coconut poonac, soyabean and gingerly meals), those of animal origin (feather meal) and those from plant material (palm press fibre and tea refuse). The reason why NCFR are under-utilised is because they contain limiting or toxic compounds and their use is considered uneconomical or harmful to animals if given in excessive amounts.

3.1 Tree fodder

This is the green forage biomass obtained from trees and shrubs. This includes leaves, twigs and other edible parts and barks of certain plant species, which are dicots and are usually perennials. By virtue of the extensive deep rooting system, these plant species are able to withstand severe drought conditions and provide quality green fodder during dry periods. Fodder trees are found growing naturally in forests, waste lands, plantations and in home gardens. Generally, they are not grown as a fodder resource, but for other purposes such as live fencing, shade, supports for climbing plants etc. However, this green biomass can be used for livestock feeding. Table 3.1 lists the fodder trees recommended for cultivation in the different agro-ecological zones in the country and the average yields of tree fodder from a single tree per year.

3.2 Advantages of use of tree fodder

Tree fodder is an economical and sustainable resource that can be conveniently used in supplementation of diets that are deficient in important nutrients. They are long lived and require little maintenance. They are generally established on sloping lands to stabilise soil against erosion because of their extensive and deep rooted nature.

Table 3.1 Species of fodder trees recommended and average fresh forage yields

Fodder tree species	Recommended zones	Defoliation frequency (months)	Fresh leaf yield (Av) kg/tree/yr
Gliricidia	MC, DZ, Co, LC, WS	3 - 4	7 - 10
Leucaena (Ipil Ipil)	DZ, Co, LCWZ	3 - 4	5 - 6
Erythrina (Dada)	HC, MC, DZ	3 - 4	5 - 7
Albizia	MC, DZ, Co, LCWZ	3 - 5	4 - 6
Calliandra	MC, Co, HC, LC	3 - 4	8 - 10

Mid country = MC, Hill country = HC, Dry zone = DZ,
Low Country Wet Zone = LCWZ, Coconut Triangle = Co

Most of these provide high quality forage and are also useful as nitrogen-rich mulch in cropping systems. Farmers do not have to purchase these valuable supplements, as fodder trees are found growing naturally or can be cultivated in homesteads or along fence lines. These fodder trees may be either legumes or non-legumes. The common fodder tree species that can provide nutritious tree fodder are Vetahira (gliricidia), Erabadu (erythrina), Ipil Ipil (leucaena), Calliandra, Murunga (drum sticks), Thiththa Sooriya (wild sunflower), Maila, Gansooriya, Wehirama (raintree), Kos (jak), Kapok, Wada (shoe flower), Kohomba (neem) and Sesbania spp. Some varieties of tree fodder contain specific limiting factors which can be harmful to animals. They are generally high in lignin and contain certain chemical substances, which may be toxic to animals if consumed in excessive amounts. Therefore, the use of fresh tree fodder as the sole feed may reduce the feed intake and also produce harmful manifestations. However, when blended with more than one single species they can contribute significantly to the nutrition of the animal. Some important characteristics of commonly used tree fodder are described below.

(a) Leucaena is a legume well known for its high nutritive value and for its similarity to alfalfa in chemical composition. It is low in sodium and iodine but high in β-carotene. Tannins in leaves and especially in stems can reduce digestibility of DM and crude protein (CP) in the rumen, but enhance their value as by-pass protein. Excess intakes may lead to metabolic problems due to the presence of toxins such as mimosine.

(b) Gliricidia too is a leguminous tree. Its leaves have high feeding value and its inclusion in low quality feeds can increase the digestibility and intake of the feed. Wilting for 12 - 24 hours before feeding has been found to increase the intake markedly and is a useful practice when palatability problems arise.

(c) Sesbania which is a legume, is readily eaten by ruminants. The DM digestibility is higher than that of most tree legumes. It contains more CP and less fibre than gliricidia and leucaena.

(d) Calliandra is a versatile leguminous shrub which is well adapted to soil conditions in most parts of the country, especially on mid-country acid soils. It is eaten readily by ruminants, no toxic substances have been found in Calliandra but it contains high concentrations of tannin which may be responsible for decreased digestibility and intake.

(e) Albizia leaves are free of toxin or excessive levels of tannin. The digestibility of immature leaves is high, but is only moderately digestible when mature, although still higher than that of mature grass. Young leaves taste bitter and therefore the feed intake is limited when offered as the whole diet, but this does not effect its value as a feed supplement.

(f) Erythrina is high in soluble CP and digestible DM. It acts as a very effective wormifuge in ruminants.

(g) Wild sunflower leaves and tender parts of stem are relished by ruminants. Digestible CP, DM and organic matter (OM) yields are highest at the 8 week stage of regrowth. No toxic substances have been identified in wild sunflower.

(h) Acasia is rather low in palatability and digestibility, but is acceptable to ruminants. It is regarded as an important forage for supplementation.

3.3 Feeding value of tree fodder

The feeding value of tree fodder varies with the species, stage of harvest and parts of the plant used. The nutritive values of the common varieties are given in Table 3.2. Though some of these varieties are commonly found in most home gardens, very little use is made of these as ruminant feed. As shown in the Tables 3.2, the leguminous varieties (Gliricidia, leucaena and wild sunflower) are rich in crude protein and therefore offer a cheap and efficient way of supplementing the nitrogen requirement of the animal, particularly in situations where animals are fed low quality fibrous feed.

Non-leguminous tree fodder varieties (Jak, kapok and Ganisooriya) are moderately rich in crude protein. As such, they could be included in the ruminant diet, in situations where grass is in short supply and the feed is of poor quality.

Wild sunflower which is commonly found in the mid and hill country areas is a very rich source of crude protein. Because of the lack of knowledge of its value as a good ruminant feed, it remains under-utilised. The other varieties of tree fodder are predominately found in dry zone areas, as they are resistant to drought. In the dry zone areas, where the dry period often extends over 6 to 7 months, tree loppings from these species could be incorporated into the diet to maintain body condition and production levels of animals. Species listed in Table 3.2 such as gliricidia, leucaena, erythrina and sesbania are rich in crude protein and can easily be grown in home gardens. They offer a very economic way of supplying protein requirements of animals.

3.4 Agro-industrial by-products (AIBP)

AIBP are by-products from industrial processing of the primary agricultural products. When compared to FCR, they are more concentrated in nutrients, have less fibre and are highly digestible. Most agro-based industries produce substantial quantities of by-products, which could be used as feed supplements. Unfortunately very little is used for this purpose at present. Examples of commonly available by-products are,

- fruit and cannery waste
- waste tea leaves
- molasses / bagasse and sugar cane tops.

Although the above-mentioned materials are available in abundance, very rarely are these used by smallholder farmers or commercial feed manufacturers as cattle and buffalo feed, due to problems and limitations associated within collection, processing and feeding.

Table 3.2 Nutritive value of leguminous tree fodder

Fodder	DM	Ash	CF	CP	Lignin	DCP	TDN
	Given as percentages						
Gliricidia (8 wks. regrowth)	-	8.4	16.8	24.3	7.7	18.5	61.2
Gliricidia (12 wks regrowth)	22.6	9.7	16.5	21.5	8.5	16.1	63.0
Leucaena leaves (8 wks regrowth)	-	7.6	12.6	26.5	8.5	20.4	52.2
Leucaena leaves and twigs (12 wks regrowth, cut 1 metre above ground)	-	6.3	21.7	14.8	6.0	10.5	56.2
Erythrina (12 wks regrowth)	19.7	4.7	39.8	14.5	-	10.2	55.0
Erythrina leaves (7 wks regrowth)	-	9.9	21.3	27.2	7.3	21.0	57.7
Sesbania leaves	19.7	7.0	21.5	21.4	-	16.1	73.4
Albizzia spp.	-	6.4	17.1	20.9	16.3	15.7	35.0
Acacia leaves and tender stems	-	10.5	14.7	17.8	-	13.0	47.5
Kapok leaves	-	14.0	19.0	12.5	12.0	-	-
Jak leaves	31.4	10.6	20.1	13.8	12.3	9.4	48.0
Palmyrah leaves	-	6.8	38.4	12	10.5	8.1	46.8
Dolichos lab lab	18.5	12.5	31.5	13.6	-	9.5	56.7
Gansuriya leaves	-	-	20.0	16.0	8.0	-	-
Neem (Kohomba)	-	-	26.0	16.0	13.0	-	-
Tamarind leaves	-	4.2	18.8	23.5	-	17.9	43.6
Maila	-	-	16.0	24.0	12.0	-	-
Wild sunflower leaves (4 wks regrowth)	-	13.3	12.7	28.1	19.3	21.8	51.4
Wild sunflower leaves and tender stem	-	12.1	14.6	16.1	7.1	11.5	54.4

CP= crude protein; CF= crude fibre

Source: Perera, A .N. F. (1992a)

3.5 Problems associated with the use of agro-industrial by-products (AIBP)

The nutritive value of fibrous feeds and limitations of use as feed for ruminants are given in the Table 3.3.

Table 3.3 Nutritive value and limitations of AIBP

Agro-industrial by-product	DM %	CP %	Fat %	Crude fibre %	Dig. %	Limitations of use
Banana Pseudo stems	12-18	5-8	1-2	25	63	High water, tannin
Manioc leaves	27	15-20	5-8	15-20	57-62	HCN content
Sweet potatoes vines	20	3-5	3-5	35-38	48-58	
Sugarcane tops	35-40	2-3	2-3	30-38	38-48	High silica and low palatability
Bagasse	88-92	1-2	1-2	45-49	25-34	High lignin; low digestibility, low CP
Spent tea leaves	87	14-17	4-6	22-26	-	High tannin
Poultry litter	90	20-24	1-2	20-40	25-32	High silica, dusty
Palm press fibre	90-94	3-5	1-2	40-46	27-32	High lignin; low CP; low digestibility
Cannery waste	10-18	5-8	1-3	18-24	54-62	High perishability and high moisture, low storage quality

DM - dry matter; CP - crude protein; Dig.% - Digestibility percentage.
Source: Perera, A. N. F. (1992)

In general, rural farmers do not regard agro-industrial by-products as suitable feed for the reason that animals sometimes reject coarse material when offered, because of low palatability and low digestibility, which results in low nutrient availability. The low digestibility is due to the high fibre content (more than 18%), low essential nutrients such as soluble sugars and proteins, minerals and vitamins and in some instances due to the presence of "anti-nutritive" factors. The use of these materials as feed requires pre-treatment to eliminate or minimise the adverse effects.

3.6 Methods of overcoming the problems associated with the use of agro-industrial by-products

3.6.1 Supplementation

In this method, the nutrients that are deficient in fibrous feeds are provided through other feed sources. For this purpose, concentrate feeds, mineral mixtures, urea, and tree fodder are generally used. Supplementation with cheap and readily available resources is the most convenient, economical and efficient method that can be practised by farmers. The details of methods of supplementation with urea are given in Chapter 4.

Table 3.4 Availability of non-conventional feed resources

Ingredients	Potential availability (MT/yr.)
Rubber seed cake	36000
Tea refuse	5000
Spent brewers grain	9000
Pineapple waste	3720
Mango seed meal	37825
Tamarind seed meal	30000
Molasses	48000
Bagasse	48146
Husk from pulses	7500
Cocoa pod husk	150

Source: Ranjhan and Chadhokar (1984)

3.6.2 Supplementation with Urea-Molasses-Mineral (UMM) multinutrient mixtures

UMM supplements can be used in feeds to provide nutrients that are deficient in AIBP in a very farmer friendly manner. This method of supplementation is very widely practised in many Asian countries and has been popularized by the SAREC/NARESA water buffalo research and development programme. The use of urea-molasses-multinutrient feed supplements is dealt with in detail in Chapter 5.

3.7 Feeding of tree fodder

Feeding of tree fodder with diets in FCR and AIDB is an economical and sustainable method of supplementation. The farmer does not have to invest or spend money to obtain this valuable supplement as this is either freely available or can be easily cultivated in homesteads along fences and other wastelands. These have certain limitations when used as feed that are specific to each variety.

Generally, some varieties of tree fodder can be satisfactorily included at substantially high levels (gliricidia, leucaena, etc.); but other varieties (e.g. Calliandra, Flemingia, etc.) may have to be limited to levels between 30-40% in the diet. In certain areas, 4-5 litres of milk per day have been obtained by feeding a combination of grass and tree fodder, without resorting to concentrate feeding.

Tree fodder could be used as a regular feed supplement to animals whose basal diet is grass and/or straw, which in most instances is poor in soluble carbohydrates, nitrogen and minerals, as shown in Table 3.2. Tree fodder is rich in soluble sugars, protein and minerals. However, there are a few limitations in feeding tree fodder, but these could be overcome by adhering to the following practices.

(i) Mixing tree fodder with grass or straw to overcome possible rejection by animals when they are given alone
(ii) Mixing several varieties of tree fodder to improve the efficiency of utilisation of feed. When mixed diets are given, the feed must be mixed thoroughly to prevent selective consumption by the animal. Further, chopping could enhance intake and

also to prevent wastage.

(iii) Tree fodder should not be fed in excess of one third of the total fibrous diet (This is true for many types of tree fodder). Excessive feeding can cause bloat and produce of toxic manifestations as some plants contain toxic substances.

The quantity of tree fodder that could be used as a feed supplement will vary with the quantity of grass and/or straw given to the animal. Table 3.4 provides guidelines for feeding of tree fodder to different breeds of cows and buffaloes. In general, the daily requirements of fresh grass of an animal is equivalent to 10% of the body weight on wet weight basis. For example, a cow weighing 100 kg would require 10 kg of grass. On dry matter basis, this is about 3% of the body weight. Therefore the straw intake of a 100 kg cow will also not exceed 3 kg. Tree fodder could replace one third by weight of fresh grass (i.e. grass to tree fodder ratio is 2:1). Tree fodder could replace

Table 3.4 Use of tree fodder in combination with grass and/or straw

Type of ruminant	Composition of diet and the quantity of tree fodder to be used in kg (fresh)
1. Zebu cow	Grass alone : 15
a) Body weight : 150 kg	Straw alone : 4.5
Milk yield : 3 litres/day	Grass + tree fodder : 10 + 5
	Straw + tree fodder : 3 + 6
	Grass +straw + tree fodder : 6 + 1.5 + 6
b) Body weight : 200 kg	Grass alone : 20
Milk yield : 6 litres/day	Straw alone : 6
	Grass + tree fodder : 13.5 + 6.5
	Straw + tree fodder : 4 + 8
	Grass +straw + tree fodder : 8 + 1.5 + 6
2. Temperate/cross bred cow	Grass alone : 20
a) Body weight : 200 kg	Straw alone : 6
Milk yield : 3 litres/day	Grass + tree fodder : 13 + 7
	Straw + tree fodder : 4 + 8
	Grass +straw + tree fodder : 8 + 2 + 8
	Grass alone : 30
b) Body weight : 300 kg	Straw alone : 9
Milk yield : 8 litres/day	Grass + tree fodder : 19.5 + 10.5
	Straw + tree fodder : 6 + 12
	Grass +straw + tree fodder : 12 + 3 + 12
3. Buffalo cow	Grass alone : 15
a) Body weight : 150 kg	Straw alone : 4.5
Milk yield : 3 litres/day	Grass + tree fodder : 10 + 5
	Straw + tree fodder : 3 + 6
	Grass +straw + tree fodder : 6 + 1.5 + 6
b) Body weight : 300 kg	Grass alone : 30
Milk yield : 8 litres/day	Straw alone : 9
	Grass + tree fodder : 19.5 + 10.5
	Straw + tree fodder : 6 + 12
	Grass +straw + tree fodder : 12 + 3 + 12

G - grass, TF - Tree fodder, S - Straw

one third of the straw in diets of an animal maintained on straw alone, (i.e. straw to tree fodder ratio is 1:2). For animals maintained on mixed diets of grass and straw, tree fodder could replace half of the forage diet (i.e. grass and straw mixture to tree fodder ratio is 1:1). For your convenience, the amounts of grass and/or straw and tree fodder recommended for feeding different types of cows and buffaloes are given in the Table 3.4.

Selected references

Devendra, C. (1992) *Non-conventional feed resources in Asia and the Pacific.* FAO-APHCA Publication No. 14. (4th Edition), FAO, Bangkok, Thailand.

Kumar, R. (1992) Anti-nutritional factors. The potential risks of toxicity and the methods to alleviate them. In: *Legumes trees and other fodder trees as protein sources for livestock.* FAO Animal Production and health Paper No. 102, FAO, Rome, Italy.

Perera, A. N. F. (1992a) Availability of tree and shrub fodder resources for ruminant livestock feeding in Sri Lanka. In: *Proceedings of the 7th International Conference of Institutions of Tropical Veterinary Medicine.* Eds. G. Tacher, Yammasubhro, Core'D Ivore. Vol. 1: 233-238.

Perera, A. N. F. (1992b) Agricultural byproducts as a feed resource for ruminants. In: *Proceedings of the Eco-friendly Commercial Activities*, MAD - Sri Lanka/NORAD, Colombo, Sri Lanka.

Preston, H.B. (1987) *Tropical animal feeding.* FAO Animal Production and Health Paper 126.

Speedy, A.W. and Pugliase, P.L. (1992) *Legume trees and other fodder trees as protein sources for livestock.* FAO Animal Production and Health Paper No. 102, FAO, Rome, Italy.

CHAPTER 4

Fibrous Crop Residues as a Ruminant Feed

S. Premaratne, M. N. M. Ibrahim, J. A. de S. Siriwardene and A. N. F. Perera

Introduction

Fibrous crop residues (FCR) are fibrous plant residues that remain after the primary crop is harvested and the economically important products are removed. They are characterised by low crude protein and mineral contents, low digestibility and high fibre and lignin complexes. The crude protein contents range from 3 to 13 percent on DM basis. This places a limitation on the value of FCR as a feed resource because generally a level of 8-10 percent crude protein is necessary to promote voluntary feed intake. These materials are also generally deficient in fermentable carbohydrates and consequently the contribution and availability of energy in the rumen is low.

Crop residues can be an important source of feed, particularly when the grazing area is limited or when there is a shortage of grass and fodder. Generally, animals in small farms tend to lose body weight and/or reproductive ability under such conditions, except in some cases where an abundant supply of crop residues is available. The use of crop residues, such as rice straw as feed for cattle and buffaloes helps to maintain animals through long dry seasons, since other supplementary feeds are not given to these animals under village small farm conditions, because of the high cost. Rice straw and other crop residues, will undoubtedly continue to play an important role in the traditional animal production systems. Crop residues could also form a very important source of roughage for dairy production in medium and large commercial dairy enterprises.

In Sri Lanka, a large amount of crop by-products and residues such as rice straw, sugarcane tops, leguminous residues, banana pseudo stems and other residues are available for animal feeding each year. These crop by-products and residues have not been fully utilised either for animal feeding or for other purposes. Sugarcane tops which form a large potential resource has not been used as animal feed in the past. In some parts of Sri Lanka crop residues are disposed by farmers by burning.

4.1 Limitations in using crop residues as ruminant feed

The availability of FCR is restricted to certain seasons of the year, usually to the crop harvesting seasons. The residues have to be collected and stored properly if they are to be used during periods of scarcity of green forage. The process of handling, transport and storage presents serious practical problems to farmers. These are associated with (1) the inability of farmers to find time and labour for this activity at a time when harvesting of the primary product is the main concern of farmers, (2) the difficulties in protecting the crop residues from exposure to rain, damp conditions and termite attack, (3) the bulky nature of the residues which entails high cost of transport and (4) the lack of proper storage facilities in the farm homesteads. These limitations

have discouraged farmers from utilising FCR in traditional feeding systems. Moreover, farmers are aware of the limitations of FCR as ruminant feeds. The low palatability and low digestibility of FCR have limited their use in feeding of animals. Further the efforts of research and extension personnel to promote supplementation of FCR with urea and urea treatment to improve its utilisation have not met with much success. However, FCR has tremendous potential as a ruminant feed as shown in Chapter I and its incorporation in feeding systems appears to be one of the promising and sustainable feeding methods available for dairy farming in the tropics, particularly in smallholder production systems.

This chapter presents information on the ways and means of improving the utilisation of FCR and recommends some appropriate farmer-friendly procedures, that can be adopted by rural farmers. The information provided here relates primarily to the use of rice straw, as it is the latest single resource in this categories available to farmers. However, the information is also applicable to other types of FCR.

4.2 Nutritional characteristics of crop residues

The chemical composition of the more important crop residues is shown in Table 4.1. As mentioned earlier, many of the crop residues are low in crude protein, high in crude fibre and low in Nitrogen Free Extract (NFE). Hence, supplementation of crop residues with protein and a readily available source of energy is a prerequisite for increasing the efficiency of utilisation of crop residues in ruminant feeding. Data on the mineral composition of crop residues is limited. However, the available information suggests that deficiencies of minerals do exist, particularly of Ca, P, Na and Cu. Supplementary feeds should therefore, include minerals to ensure improved utilisation in the rumen. When crop residues are fed with green forage like grasses, legumes and tree fodder, it is suggested that straw and green forage and straw and tree fodder should be mixed in the ratio of 1:4 and 1:2 respectively for animal maintenance and growth.

4.3 Rice straw

Rice straw is the most abundant crop residue available in Sri Lanka. It is unique in several ways in that the stems are more digestible than the leaves, unlike other straws, where the opposite is true. Rice straw contains much more silica (12-16%) and less lignin (6-7%) than other straws, which contain 3-5% silica and 10-12% lignin. For this reason, rice straw should be cut as close to the ground as possible, for feeding of livestock. According to scientists, although 30% of the silica in rice straw is absorbed and excreted in the urine, urinary calculi are not generally a serious problem. Rice straw can be upgraded in feeding value to a level equivalent to that of medium quality hay by application of appropriate processing methods.

4.4 Factors affecting quality of rice straw

The nutritive value of straw and other crop residues varies greatly depending on the plant component, species, cultivar, maturity, application of fertiliser, weathering and the harvesting height. In general, the feeding value declines as the plant matures. It is

well known that the feeding value of straw from short improved varieties of rice is much higher than those from tall varieties. Rice stubble is said to have a higher digestibility than rice straw, although it is generally accepted that the tops of plants are more digestible than the lower parts. Thus different cutting heights may lead to a variation in quality. The method of threshing and storage conditions can also effect quality as a result of the removal or the fragility of parts of leaves. Any type of processing of crop residues is likely to change the feeding value of the material. In general, straw from unirrigated fields is said to have a higher feeding value than straw from irrigated fields.

Table 4.1 Chemical composition of crop residues expressed as percentage of dry matter.

Crop residue		Crude protein	Crude fibre	Nitrogen free extract	Ether extract
1. Rice	Straw	3.25	33.65	47.91	1.03
2. Maize	Stalk	3.57	33.18	51.92	0.79
	Cobs	2.06	36.4	57.89	0.78
3. Sorghum	Stalk	3.80	30.46	55.42	1.73
	Straw	6.60	30.40	52.70	1.20
4. Kurakkan	Straw	3.67	35.92	51.38	0.92
5. Cowpea	Hay	15.37	34.84	35.44	1.09
6. Green gram	Straw	12.54	24.44	47.04	1.03
7. Black gram	Straw	10.66	28.82	46.73	0.44
8. Ground nut	Stalks	15.01	27.59	43.70	2.88
9. Sugar cane	Dried leaves	2.64	37.18	52.78	1.36
	Green leaves	5.90	36.30	47.00	1.70
	Tops, post-ripe stage	4.50	31.90	48.10	3.00
10. Manioc	Fresh leaves	24.10	26.00	39.90	5.00
11. Sweet potato	Fresh vines	21.90	15.00	41.70	3.40

Source: Perera, A. N. F. (1992); Chadhokar, P. (1982)

4.5 Nutritive value of rice straw

The nutritive value of roughage is dependent on several factors such as the nutrient composition of the feed, the voluntary intake of the feed, which reflects the amount of nutrients consumed, the digestibility of the feed consumed and the efficiency of utilisation of the available nutrients in metabolism.

All these criteria, however, cannot be applied in measuring the nutritive value of crop residues because of the inadequacy of information. Generally, assessment of quality is based on chemical composition and digestibility. It should therefore be borne in mind that in the absence of adequate information relating to local crop residues, the use of data from other sources may not give an accurate assessment. But for practical purposes, assessments based on nutrient composition and digestibility can be considered adequate.

The composition of rice straw in comparison to unfertilised mature and immature Guinea grass (*Panicum maximum*) is given in Table 4.2. As shown in the Table, the

water content of rice straw is 8-12% compared to that of mature or immature Guinea A grass (71 - 73%). As water is a nutrient that is important to all forms of life, the first limitation in rice straw is its low water content. Therefore, animals fed straw should have access to drinking water at all times. Inadequate provision of water could create problems in the dry zone during the dry season, when straw feeding is practised. Rice straw is also low in most of the important mineral constituents like Ca and P. As the crude protein content of rice straw is generally low (below 5%), the importance of supplementation of straw with protein needs hardly be emphasised. Research has shown that mineral imbalances and the low crude protein content in rice straw could be easily corrected by providing suitable supplements. Although rice straw is rich in cellulose and hemi-cellulose, which are potential sources of energy for ruminants, the digestibility of these components is low. This could be mainly due to protective or masking effect of lignin, silica and other compounds present in close association with these components in the cell wall matrix.

Table 4.2 Chemical composition of young and mature unfertilised Guinea grass (*Panicum maximum*, Ecotype A) as compared to rice straw.

	Guinea A[1]		Rice straw[2]
	3 weeks	9 weeks	
DM %	22.9	28.7	88 - 92
Ash % DM	11	10.5	7 - 14
CP %	12.4	7	4 - 5
NDF %	66.5	74.4	72 - 81
ADF %	38.7	50.9	45 - 52
Cellulose %	29.7	39	36 - 43
HC %	27.9	23.5	23 - 34
Lignin %	6.1	9.3	5 - 7
SiO_2 %	3.5	3.8	5 - 9
Ca mg/g	4	4.5	1.9[3]
Mg mg/g	3.2	3.6	1.0[3]
P mg/g	1.9	1	0.7[3]
Na mg/g	0.4	0.1	0.5[3]
IOMD %	57.1	35.1	35 – 45

1. Pieris, H. (1985)
2. Premaratne, S. (unpublished data)
3. Ranawana S. S. E. (1986)

4.6 Methods to improve the nutritive value of rice straw

The digestibility or bio-degradability of rice straw could be improved by pre-treatment. Several treatment processes are technologically possible but not economically feasible. Treatment processes can be grouped into physical, chemical physico-chemical and biological methods. Farmers require cheap, safe, technologically simple and effective methods of treatment. As such, the discussion hereafter will be mainly focussed on treatment methods that are suitable for Sri Lanka and other developing countries. However, the methods that are of little practical use are also

presented here, but very briefly for the completeness of the review. Broadly, the methods available could be grouped into two categories:

(a) Physical and chemical treatment of straw
(b) Supplementation of the straw-based diets to rectify deficiencies

4.6.1 Physical and chemical treatment

(a) Grinding and pelleting: Grinding and pelleting may increase voluntary intake but frequently depresses digestibility. Increased rate of passage due to grinding causes higher intakes and results in lowered digestibility. The economic constraints imposed by the cost of processing and pelleting have discouraged the use of these methods in most countries in Asia.

(b) Chopping: Coarse crop residues could be chopped into 8-10 cm lengths, before feeding. Chopping alone will not improve the intake because of the coarse nature of the material. Soaking for 6-8 hours before feeding tends to improve the texture of the chopped material. One disadvantage is that some of the water soluble nutrients, (sugars and proteins) may also be lost.

(c) Soaking and wetting: Dry matter losses of between 8-14% has been reported when rice straw is soaked for 3 days. This due to the removal of the soluble cell contents. Reports on the affects of soaking on digestibility are contradictory. The general consensus appears to be that soaking does not seem to affect the nutritive value and digestibility of rice straw. In parts of India, soaking of straw prior to feeding is a traditional practice, because of the belief that the removal of oxalates from straw has a beneficial effect.

(d) Steaming and irradiation: Steaming under pressure brings about chemical changes such as the cleavage of bonds between cell wall constituents and lignin. Steaming increases the digestibility and the moisture content of straw but decreases the dry matter due to volatilisation. Irradiation of low quality straw can severely depress the digestible energy value. Both these treatment procedures are costly and therefore have no application in small scale farming systems found in Asia.

(e) Chemical treatment: The objective of chemical treatment is to increase the intake and digestibility of fibrous feeds by breakdown of complexes of lignin and cell wall carbohydrates. This can be done with caustic soda (NaOH), caustic potash (KOH), burnt lime (CaO) or urea [$(NH_2)_2CO$]. Of these chemicals, the cheapest, least hazardous and the most convenient to use is urea. This method was popularised few years ago in Sri Lanka. However, the adoption rate had been disappointingly low. This method is widely used in many countries to improve the quality of fibrous feeds. The chemical generally used is fertiliser grade urea.

(f) Sodium hydroxide treatment: Until the mid 1970s, much of the reported work has been on the use of sodium hydroxide. Although sodium hydroxide is very effective in increasing the digestibility of cereal straw, limitations such as high cost, corrosiveness,

environmental pollution and health hazards makes it less attractive for use in developing countries.

(g) Calcium hydroxide treatment: Calcium hydroxide is the cheapest hydroxide available in most of the developing countries. Due to its low solubility in water there seems to be a need to use large quantities of water or to use a long treatment period (about 100 days). A development of a suitable methodology for the use of calcium hydroxide, which is simple to apply and easy to handle has not been systematically researched as yet.

(h) Ammonia treatment: Compared to ammonium hydroxide (aqueous ammonia), anhydrous ammonia (ammonia gas) has been preferred to treat cereal straws. By use of this method, it is possible to treat about 5 - 10 MT of dry and baled straw at a time. The treatment time depends on environmental temperature. Although, considerable increases in digestibility and intake could be achieved by ammonia gas treatment, its use in developing countries is limited due to high cost and unavailability of this chemical at farm level.

(i) Urea treatment: The use of urea as a source of ammonia has been receiving attention during the past few years. A substantial amount of information has become available in Asia. Of the various chemicals discussed, urea seems to be most appropriate for use in developing countries due its low price, availability at farm level and also because the farmers are familiar with this chemical.

Research conducted in Bangladesh, Philippines, Thailand, Indonesia, India and Sri Lanka shows that the crude protein content of rice straw could be increased at least two fold, the digestibility by 10-15 units and more importantly an increase in intake of up to 40% could be achieved by treating straw with urea-ammonia. It is obvious that urea-ammonia treatment is the most applicable method presently available for small farmers in Sri Lanka and many other tropical countries.

4.6.1.1 *Method of urea treatment*: Chopped straw or stover (straw may be used without chopping) is spread on a cemented floor or on a polythene sheet. A 4% urea solution is sprinkled using a watering can or a tin with a perforated base. Four percent urea solution is made by dissolving 40g of urea in 1 litre of water. For treatment, the ratio of straw:water should be 1:1. After treatment, the treated straw must be covered with a polythene sheet to prevent the escape of ammonia gas formed from urea. Another convenient method of preventing the loss of ammonia, is by packing the treated straw into polysack bags and tying the open ends. The bags should be covered with a large polythene sheet and stored for 7 days to allow time for the urea to react with the straw. The straw that is treated on day 1, will therefore be ready for feeding on day 7.

After 7 days of storage, the polythene cover is opened to expose the quantity of straw required for the day. Prior to feeding, the treated straw is allowed to remain exposed for half to one hour to facilitate the liberation of excess free ammonia. Urea treated straw must be introduced to animals gradually, offering 10% of the total feed and daily

increasing the amount offered so as to allow time for the animal to adapt to the new feed over a period of 7 to 10 days.

4.6.2 Supplementation of the straw based diets

This method will help to provide nutrients that are deficient in rice straw and stover. Supplementation will improve the quality of the ration in the following manner.

(a) By optimising the rumen nutrient balance by providing the limiting nutrients such as ammonia nitrogen, soluble sugars and minerals to improve digestibility (***catalytic effect***)

(b) By providing deficient nutrients to restore the nutrient balance in low quality feed (***supplementary effect***)

Supplementation does not change the structure of the fibrous feed, but facilitates its utilisation. Supplementation is an easier method for the farmer to practice than chemical treatment. Moreover, the feed can be harvested and offered to the animal on the same day. The type of supplement to be fed will depend on the nutrients that are deficient in the basal feed.

4.6.2.1 *Type of supplements:*

1. Urea to provide rumen ammonia nitrogen
2. Urea-molasses-mineral multinutrient (UMMM) mixture to supplement rumen ammonia nitrogen, soluble sugars and minerals
3. Concentrates to supplement protein, carbohydrates and minerals
4. Tree fodder to provide deficient proteins and soluble sugars

(a) Supplementation with urea

Urea could also be used as a supplement for fibrous feed. This could be done by sprinkling urea solution on fibrous feeds or incorporating urea in the concentrate feed mixture. Urea supplementation has proved to be equally effective as urea treatment when fed with fibrous feed, but the level of urea used must not exceed more than 1.0-1.5% of the total feed on dry matter basis. This could be achieved by sprinkling 1 to 1.5% urea solution (10-15 g of urea dissolved in 1 litre of water) at the rate of 1 litre per 1 kg of straw. Urea sprinkled straw could be fed after 2-3 hours of sprinkling. One must make sure, as with the feeding of urea treated straw, that a source of readily available energy, such as molasses must be given, and that urea supplemented straw must be gradually introduced to the animal.

It is important to remember that urea, if given in excess could lead to toxicity. In the event that excess urea is consumed, the animal will exhibit symptoms of ammonia toxicity such as purple or blue colouration of the mucous membranes of the mouth, muzzle and eyes. In such a situation, administer 1-1.5 litres of vinegar orally.

(b) Supplementation with Urea-Molasses-Mineral Multinutrient mixtures

Urea-molasses-mineral multinutrient (UMMM) mixtures provide complete supplementation of nitrogen, energy and minerals. This is widely used in many countries and is becoming popular in Sri Lanka. Details of UMMM and its use are provided in Chapter 5. This is made by mixing molasses (35-45%), rice bran (30-40%), urea (10-12%) and minerals (3-5%). The exact composition of the UMM multinutrient mixture and the form (bricks or flakes) could be varied to suit the feeding practices under different production systems.

(c) Supplementation with concentrate feed

This is the most common method of supplementation. At the present time, this has become an uneconomical practice due to the high price of concentrates in relation to the low price received by the farmer for milk. Although commercial concentrate feeds are readily available in the market place, farmers often prefer to prepare concentrate mixtures using commonly available ingredients, such as coconut poonac and rice bran. Even when mixed on farm, concentrates are the most expensive feed available for milk production. As a general rule, 1 kg of concentrate is given for maintenance and thereafter, an additional 1 kg is provided for every 2 litres of milk produced.

(d) Supplementation with tree fodder

Feeding of tree fodder is an another economical and sustainable method of supplementation. The benefits of supplementation of low quality feeds was dealt with in detail in section 3.7 of Chapter 3.

Generally, tree fodder can be satisfactorily included at a level of between 30-40% in the diet. In certain areas, 2-3 litres of milk per day is obtained by feeding a combination of grass and tree fodder, without resorting to concentrate feeding.

4.7 Practical guidelines for use of rice straw as ruminant feed

4.7.1 Collection and storage of rice straw

Rice straw is a seasonally available feed resource. In certain areas of Sri Lanka, straw is produced twice a year. Straw must be collected as soon as possible after the rice is threshed, properly dried before storing, to preserve the keeping quality and stored in a manner that will protect it from rain or dampness. Wet straw allows moulds to develop and soon becomes unsuitable for livestock feeding. Mouldy straw is unacceptable to ruminants and if consumed, it may lead to certain metabolic disorders or toxicities due to presence of mycotoxins. Well-dried straw when suitably stored can be kept for nearly 2 years without deterioration of its original nutritive value or acceptance by the animal. Well-dried straw can be made into bales or loosely stacked in a hay barn or similar structure. Straw must be stored in a manner that will allow proper cross-ventilation, or heaped on a rock or on woody platform as in the "Kolaya" method. When stored unprotected in the open, precautions must be taken to prevent termite attack, by stacking straw on an elevated base.

It is important to remember the following points:

- Collect and properly store an adequate quantity of straw for drought feeding.
- Treat straw daily to meet one day's requirement.
- Do not feed refusals on the next day.
- Do not exceed the concentration of urea solution more than 4% when urea treatment is practised.
- Do not feed treated straw as soon as the polythene covering is opened. Animals may refuse to eat.
- Continue feeding of treated straw until grass becomes available and withdraw the feeding of treated straw gradually as grass becomes available.
- When feeding urea treated straw, it is important to provide a readily available energy feed like concentrates. This should form at least 10% of the total basal ration (100g of concentrate per kg of dry straw). For cows producing above 2-3 lit/d, a supplementary feed must be given at the rate of 1 kg for maintenance plus 1 kg of concentrate for every 2 litres of milk. But as concentrates are expensive, fresh grass rich in soluble sugars could be given with urea treated straw, whenever possible, in order to reduce the feed costs. The alternative is to feed supplements such as molasses when urea treated straw is fed.

4.8 Cost comparison of different methods of supplementation[1]

(a) Cost of feeding a cow with urea treated straw

Straw is assumed to be cost free	
Cost of urea	*= Rs. 15.00 per kg*
Cost of polythene per day	*= Rs. 1.26*
Requirement of urea for 1 ton of straw	*= 40 kg*
Cost of urea to treat a ton of straw	*= Rs. 15.00 x 40 (Rs. 600.00)*
The daily rice straw requirement of a cow	*= 3.5% of body weight*
Total weight of straw for a 300 kg cow (3.5x 3)	*= 10.5 kg straw/day*
No. of days to consumes 1 ton of straw (1000/10.5)	*= 95 days*
Feeding cost/day (600.00/95)	*= Rs. 6.30*
Total cost including cost of polythene	*= Rs. 7.56 (6.30 + 1.26)*

[1] Cost estimates are based on the 1998 prices and the purpose of the exercise is to emphasis the economic advantages of different methods of supplementation. Labour cost is not taken into consideration as it is available at no cost to the farmer.

(b) Cost of feeding a cow with urea sprinkled straw

Strength of urea solution	*= 2%*
Urea requirement for 1 ton of straw	*= 20 kg*
Cost of urea	*= Rs.15.00 x 20 (Rs.300.00)*
No. of days to consume 1 ton of straw	*= 95 days*
Feeding cost per day	*= (300.00/95) = Rs.3.15*

(c) Cost of feeding a cow with urea-molasses-mineral mixture and rice straw.

For a lactating cow consuming the following feed, provide the amounts of UMMM shown alongside.

(i)	*Good quality forage*	*= 0.5 kg of UMMM/day*
(ii)	*Medium quality forage and straw*	*= 0.8 kg of UMMM/day*
(iii)	*Poor quality forage and straw or mature grass*	*= 1.0 kg of UMMM/day*

Cost of 1 kg of UMM = *Rs. 8.00*

Cost of UMMM per day for each of the above feeding systems:

(i) *0.5 x 8 = Rs. 4.00*
(ii) *0.8 x 8 = Rs. 6.40*
(iii) *1.0 x 8 = Rs. 8.00*

Note: Feeding of UMMM supplements would be sufficient to meet the nutritional requirements of a cow giving less than 5 litres of milk per day. In the case of cows that produce more than 5 litres per day, UMM feed supplement could replace 30-40% of the quantity of concentrates provided on the basis of level of production.

Selected references

Chadhokar, P. A. (1982). *Recommendations of tropical forage development.* UNDP/FAO tropical pasture development project. Sri Lanka/I.D.A. Dairy Development Project, Gatembe, Peradeniya.

Ibrahim, M. N. M. (1983). Physical, chemical, physicochemical and biological treatment of crop residues. In: *The Utilization of Fibrous Agricultural Residues*. G. R. Pearce (Ed), Australian Government Publishing Services, Canberra, Australia, pp: 53 - 68.

Ranawana, S. S. E. (1986). Mineral supplementation of straw based diets. In: *Rice Straw and Related Feeds in Ruminant Rations.* M. N. M. Ibrahim and J. B. Schiere (Eds), Straw Utilization Project. Pub. No. 2, Department of Tropical Animal Production, Wageningen Agricultural University, The Netherlands.

Peiris, H. (1985). Management practices to improve the yield and nutrition value of unfertilized *Panicum maximum* (Ecotype A) in the mid country wet zone of Sri Lanka. *M.Phil Thesis.* Postgraduate Institute of Agriculture, University of Peradeniya, Sri Lanka.

Perera, A. N. F. (1992). Agricultural byproducts as a feed resource for ruminants. *Proc. Eco-Friendly Commercial Activities.* MAD - Sri Lanka/NORAD, Colombo.

Preston, T.R. (1986) *Better utilisation of crop residues and by-products in animal feeding: research guidelines.* FAO Animal Production and Health Paper No. 50/2, FAO, Rome, Italy.

SundstΦi, F., Coxworth, E. and Mowat, D. N. (1978). Improving the nutritive value of straw and other low quality roughage by treatment with ammonia. *World Animal Review*, 26: 13-21.

CHAPTER 5

Use of Urea-Molasses-Mineral (UMM) Multinutrient Feed Supplements in Ruminant Feeding

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Introduction

As discussed in Chapter 1, dairy buffaloes and cattle require good quality forage in sufficient quantities, as well as supplementary feeds rich in proteins, soluble sugars and minerals, to enable them to express their full potential to grow, reproduce and produce milk.

Traditionally, dairy buffaloes and cattle have been reared on natural pasture, often supplemented with tree fodder that is available in the locality, particularly when good quality grass is not available in adequate quantities. The quality of grasses and fodder varies widely from season to season depending largely on the rainfall pattern. During dry periods, both the quality and quantity of forages decline. In such situations, the immediate affect that is evident in dairy animals is a decline in milk yield and body condition. Farmers with good milking animals have, under such circumstances, learnt to supplement the available forage with either coconut poonac or rice bran or a mixture of coconut poonac and rice bran (concentrates) to prevent a decline in milk yield. But generally, these concentrates are fed in limited quantities, because of the high cost, compared to the price received for milk at the farm gate. The ignorance on how to introduce alternative feed supplements often leads to underfeeding animals and as a result, the nutrient requirements of animals in terms of protein, energy, minerals and vitamins are not met. This leads to a decline in milk yield, body condition and fertility.

These limitations could be overcome by adopting more economic and strategic feeding regimes, utilizing low cost feed resources such as crop residues (e.g. rice straw) and agro-industrial by-products (e.g. molasses in combination with urea). In order to understand how these products could be used in ruminant feeding, a sound understanding of the processes of rumen microbial digestion of feeds is essential. Chapter 2 has described the principles of rumen microbial digestion and factors which limit rumen microbial digestion. In Chapter 4, the value of supplementation, particularly with Urea-Molasses-Mineral multinutrient has been mentioned and Chapter 3 has described the way and means of overcoming some of the limitation of ruminant feeding by using tree fodder. Therefore, the description given below is limited to the use of Urea-Molasses-Mineral (UMM) supplements in overcoming nutritional deficiencies of cattle and buffaloes.

5.1 The role of UMM multinutrient mixture as a feed supplement

As discussed in Chapter 2, one of the important factors that influence microbial growth and activity in the rumen is the rumen ammonia concentration. The principles

relating to the maintenance of a stable rumen environment to ensure maximum microbial growth and activity were dealt with in Chapter 2. Sub-optimal conditions in the rumen environment are produced when the feed is either inadequate or low in quality. In such situations, the use of urea-molasses-mineral-multinutrient (UMMM) supplements either as blocks (UMMB) or granules (UMMG) or paste (UMMP) offers a unique way of meeting the nutrient deficiencies in poor quality feeds. UMMM supplements provide nitrogen in the form of urea, a readily available energy source in the form of rice bran and molasses and also minerals. Of the three different forms mentioned above, the use of UMMM in the form of a block, available to the animal at all times, ensures a steady supply of nutrients for maintenance of a stable rumen environment.

UMMM supplements are particularly useful when the quality of forage supplied to animals is poor and/or inadequate. The steady intake of nutrients from this source helps to maintain microbial activity at an optimum level, which in turn helps to increase the (1) efficiency of microbial fermentation (2) rate of passage of digesta out of the rumen and (3) increase feed intake.

5.2 Composition of UMM multinutrient feed supplements

UMMM supplements are made up of several basic ingredients namely urea, rice polish, molasses and mineral mixture. The composition of UMM supplements is shown in Table 5.1. Depending on the levels of limiting essential nutrients in the feed offered to animals under a particular feeding management system, additional ingredients such as fish meal, coconut poonac and soyabean meal can be incorporated. As mentioned earlier, anthelmintics and other feed additives can also be incorporated in situations when slow release of drugs or additives need to be introduced in a manner that will ensure the maintenance of the required concentrations within the animal system. Cement is incorporated as a binder, when the supplement is produced in the form of a brick (UMMB).

5.3 Production of UMMM supplements

The items of equipment required for the small scale production of UMM Multinutrient feed supplements are a mixer, which can be a concrete mixer or a horizontal mixer, a mould or press for making bricks and a weighing scale. Additionally, facilities for drying the product, for which wooden or metal racks or adequate floor space are required. The ingredients should be weighed separately in amounts required to produce the pre-determined quantity of the supplement. The urea and mineral mixture are first premixed with about 5-10 times the combined weight of rice polish. The balance rice polish is placed in the mixer and mixed for 2-3 minutes, gradually introducing the premix of rice polish, minerals and urea. The molasses is then poured into the mixer and allowed to mix for 8-10 minutes. Thereafter, as the mixing proceeds, the cement is added and mixed for a further period of 5 minutes. This mixture is made into bricks using a mould or press and left to dry. Drying takes about 7-10 days depending on weather conditions.

The UMM Multinutrient supplement can be produced either in the brick form (UMMB) or as granules (UMMG) or as a paste (UMMP). UMMB is a convenient form to feed animals reared under intensive management. The maximum benefit through feeding UMMM can only be obtained if it is given as a free choice lick. This will ensure the maintenance of the rumen ammonia concentration at an optimum level throughout the day and also facilitate the continuous digestion of coarse fibrous feed.

Table 5.1 Composition of UMMM feed supplements

Ingredient	Proportion
Urea	10-12% (should not exceed 10%)
Molasses	30-45% (may vary with the quality of molasses)
Rice polish	30-50% (used as a source of energy and as a filler)
Mineral mixture	03-05% (composition will depend on the known mineral deficiencies in the basal feed)
Fish meal	5-10% (optional; may be needed only for high producing animals)

Note: For UMMM blocks, cement is used at 10-12% replacing rice polish. Also note that the composition of the UMMM preparation may vary with the type of production system.

In situations where feeding of UMM Multinutient feed supplement as a lick is not feasible, for example with animals reared under extensive management systems, UMM granules (UMMG) can be fed mixed with concentrates and fed two or three times a day. Although this is not the best method, it will nevertheless help to maintain the rumen ammonia concentration at an adequate level, although only for limited periods after UMMG is consumed. If UMMG is not available, UMMB can be chopped into small pieces and fed in divided doses 3 or 4 times over a 24 hour period. Alternatively, it could be given mixed with concentrates such as rice polish or coconut poonac at milking time.

5.4 Methods of using UMM Multinutrient feed supplements for optimum benefits

UMM feed supplements are produced in two forms to suite different management systems.

(a) To obtain maximum benefits from UMM feeding, animals should have access to UMM as a lick to consume at will. The animal will only consume sufficient UMM adequate to meet the deficit in the limiting nutrients, so as to maintain the optimum level of ammonia in the rumen, which is between 180 and 200 mg/l of rumen fluid. In situations where the use of the UMM block is not convenient to the farmer, the granular form or a portion of the block can be crushed and mixed into concentrate feed or poonac. UMM supplements should not be fed in excess of 1 kg per animal per day.

(b) The maximum benefits from feeding UMM supplement can only be obtained if the animal has free access to the UMM feed throughout the day, to ensure the maintenance of optimum rumen conditions, that will promote continuous microbial activity. When sufficient good quality feed is available to the animal, the voluntary intake of UMM will decline. Thus when UMM is available as a block, the animal will regulate the intake depending on the quality of the feed offered.

UMM feed formulations can be used in two ways:

(a) For cows yielding less than 5 litres of milk per day: up to 1 kg of UMM can be fed in place of concentrate feed, together with forage and/or crop residues (total replacement).

(b) For cows yielding more than 5 litres per day: 1 kg of UMM can be fed as a supplement, to replace 30-50 percent of the concentrate feed in the daily ration (partial replacement).

In both the above situations, rumen digestion of fibrous feed is enhanced as described earlier and hence the intake of fibrous feed will increase. Therefore, it is important to remember that animals must be given more than their usual requirement of fibrous feed to obtain optimum benefits from UMM feeding. Remember that UMM is not a replacement for fibrous feeds.

5.5 Recommended feeding method

(a) The maximum quantity of UMM feed supplement for an adult cow recommended per day is 1 kg. This limit should not be exceeded.

(b) If the block form (UMMB) is used, it should be placed in a wooden box and kept in a suitable place where the animal has easy access for licking when required. If the animal starts to bite or eat the UMMB, remove it for a few hours and replace it again. Ensure that the animal does not consume more than 1 kg/day.

(c) If the granular form (UMMG) or chopped UMMB is used, feed up to 1 kg/day in divided quantities at milking time, mixed with concentrate.

(d) Whatever the form of UMM feed supplement that is used, more than adequate quantities of fibrous feed and water must be provided. Ensure that fibrous feed is available at all times in the feed trough or in the grazing area and that animals have easy access to water.

5.6 Some important points to remember when UMM is fed to the cows

(a) UMM feed supplements should not be given to animals below 3 months of age.

(b) UMM should be introduced gradually to allow time for adaptation to the new feed. Animals may sometimes not consume UMMB when first introduced. Give time to animals to start licking the block.

(c) When UMM feed supplements are given to animals, water must be given *ad-libitum.*

(d) UMM feed supplements, whether as UMMB or UMMG, must be protected from rain, dust, dung and urine.

Selected references

Jayasooriya, M.C.N. (1993) Use of crop residues and agro-industrial by-products in ruminant production systems in developing countries. *Animal Production in Developing Countries.* Occasional Publication. No. **16,** 47-55.

Leng, R.A., Preston, T.R., Sansoucy, R. and Gorge Kunju, P.J. (1991) Multinutrient blocks as a strategic supplement for ruminants. *World Animal Review* **67,** 11-19.

Preston, T.R. and Leng, R.A. (1987) *Matching ruminal production systems with available resources in the tropics and sub-tropics.* Preambul Books, Armidale, Australia.

Sansoucy, R. (1995) New developments in the manufacturing and utilisation of multinutrient blocks. *World Animal Review,* No. 82, FAO, Rome, Italy.

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CHAPTER 6

Ration Formulation - Principles and Practice

M. N. M. Ibrahim, H. Abeygunawardena and J. A. de S. Siriwardene

Introduction

The preceding chapters dealt with the principles of nutrition and the resources available to farmers to develop feeding systems to suit the different management systems practised in the country. The success of a dairy enterprise hinges on the ability of the farmer to develop economic feeding systems to meet the nutrient requirements of animals, based on the cheapest feed resources available in the locality. In this exercise, it is necessary to relate the nutritional characteristics of the available feed resources to the requirements of the animals. This chapter provides the necessary information that will enable veterinarians to advise extension workers and farmers how to formulate feed rations, utilising the basic knowledge provided in the preceding chapters.

The two major sources of feed are roughage and concentrates. The cheapest source of feed is roughage (i.e. grass, fodder and crop residues). For this reason, maximum use must be made of the grass, fodder and crop residues available in and around the farm. Concentrates and other supplements should be used mainly to provide the nutrients necessary to meet any deficiencies in nutrients available to the animal from the roughage offered. The nutrients required by animals are energy, protein, water, minerals and vitamins. All these nutrients are important to keep the animal in good body condition. The energy and protein components in the feed are not completely digested and the undigested fraction passes out in the dung. The amount of available energy in the feed is termed the Total Digestible Nutrients (TDN) and the amount of available protein is termed Digestible Crude Protein (DCP). To simplify matters, the nutrient requirements of animals will be expressed here in terms of TDN and DCP. The animal has a limited intake capacity and for this reason the daily TDN and DCP requirements of the animal should be contained in a ration that is not in excess of the intake capacity of the animal.

It is common knowledge that most smallholder farmers do not feed their animals adequately. As a result, productivity falls below the potential of the animal, and in the case of dairy animals the milk yields may fall below the expected levels. Fibrous feeds are not easily digested and are generally deficient in one or more of the important nutrients. This problem is more acute during the dry season when animals are fed on poor quality feed. Under-nutrition can drastically increase in nutrient deficiencies.

Water, energy, protein and minerals must be provided in adequate, balanced amounts if optimum results are to be achieved. The requirements of the animal for these nutrients depend on whether the animal is;

Idling (maintenance requirements)
Growing (growth requirements)
Pregnant
Producing milk or a
Working animal

Data available from nutrient requirement tables in books and scientific papers should be treated as a guide in establishing feeding regimes and should not be condsidered as absolute values. They do not replace the expertise of the farmer in matching the nutrient intake with the expected performance of the animal. Nutrient requirement data can be used as a starting point in formulation of a diet and also to estimate the feed requirements for any period of time. The first step in formulating a diet is to establish guidelines as to how much of each of the various nutrients is required to be supplied as nourishment to the animal, in amounts adequate to meet the requirements for maintenance, growth, production and reproduction. In order to feed the animal according to its requirement, one should know:

(a) the nutrient requirements of the animal,
(b) the nutrient contents of the available feeds, and
(c) the amount of feed the animal can consume

6.1 Nutritive requirements of animals

The nutritive requirements of animals vary with the physiological status. The body weight, milk yield and pregnancy status has a bearing on the nutritive requirements. The daily requirement can be expressed in terms of total digestible nutrients (TDN) and digestible crude protein (DCP). These requirements must be provided to ensure optimum production from the animal. The requirements of TDN and DCP of animals can be obtained from published Feeding Tables. A standard table giving the TDN and DCP requirements of buffalo cows with a body weight of 350 kg, producing from 1-10 litres of milk per day containing fat varying from 4 to 8%, is given in Table 6.1

6.2 Nutritive values of feedstuffs

The nutritive value of a feed is usually assessed by the amounts of nutrients it contains. Feeds can be generally classified as roughages and concentrates. Roughages include grasses (natural, improved), creepers (climbers), legumes (siratro, centrosema), leguminous (glyricidia, ipil ipil) and non-leguminous (jak, mango) trees and shrubs, agricultural (straws, stover) and agro-industrial (soya hulls, bagasse) by-products. Concentrates include coconut poonac, rice bran/polish etc. Rather elaborate information on the nutritive values of locally available feedstuff in Sri Lanka is given in Ibrahim (1988a and b).

The biological value of a feedstuff is determined by the:
Dry matter content
Protein content
Energy content
Digestibility

Amount and type of minerals
Amount and type of vitamins

This information is also available from feed composition tables and scientific publications. Table 6.2 gives the composition of some selected feeds generally available to farmers.

6.3 Intake of dry matter

The amount of nutrition that an animal receives depends on 2 factors:

(a) the nutrient content of the feed and
(b) the feed intake

It is relatively easy to measure the quality of the feed offered, but it is difficult to assess how much of the feed offered will be consumed, particularly if the animal obtains part of its ration from grazing or browsing. One of the most important skills in animal husbandry is to be able to develop a management system where animals will obtain a part or the entire nutritional needs by eating more of cheap, low quality feeds rather than expensive feeds. In order to do this, one should have an idea of the consumption pattern of different forms of feed, even though it may not be possible to know exactly how much an animal will eat. Animals have two sets of competing feelings about feed;

(a) the desire to start eating (appetite) and
(b) the decision that enough has been eaten (satiety)

In general, how much a cow will eat depends on its body weight, the milk yield and the type and quality of feed. A higher intake of concentrates will lower the consumption of roughage, even if the roughage is of high quality. If the quality of the roughage is average or low, concentrates may slightly increase the intake of the roughage. In order to prevent disorders in the functioning of the rumen, it is advisable to ensure that at least a third of the total DM in the daily ration is made up of roughage.

6.4 Protein

The protein requirements of cows and the protein content of a feedstuff are expressed as (a) grams of crude protein (g CP) or (b) grams of digestible crude protein (g DCP) per Kg of feed. The protein requirements of cows can only be met from the nitrogenous compounds in the feed (true protein and non-protein nitrogen). A shortfall in nitrogenous compounds in the feed cannot be compensated by substitution of carbohydrates (CHO) or other substances.

6.5 Energy

Energy is supplied by the organic matter in the ration, through CHO, protein and fats. The cow only uses protein to obtain energy when there is not enough CHO and fat in

the ration to meet the energy requirements or when protein is fed in excess of the requirement. 'Protein for energy' should be avoided because protein is usually expensive. The amount of energy that a cow needs and the amount of energy that a feedstuff supplies can be expressed in several ways. For simplicity, the oldest and the most common system, namely the Total Digestible Nutrient (TDN) system is used here.

In the TDN system:

(a) 1 kg of CHO is assumed to liberate 4000 kcal of energy.
(b) 1 kg of crude protein liberates 4000 Kcal of energy and
(c) 1 kg of liberates 9000 Kcal of energy.

6.6 Concentrate feeds

Concentrates are feeds rich in available nutrients such as energy, protein, minerals and vitamins that are essential to the animal. It is important to remember that concentrate feedstuffs that are by-products of industrial processes, such as rice bran can vary in quality. For instance, the TDN value of rice bran can vary from 40% to 60% depending on the type of milling process employed. The variation in quality is a function of the efficiency of separation of hulls from rice polish. Concentrate feeds are generally relatively expensive and the extent of their use should be determined on the basis of the cost effectiveness of the level of inclusion in feeds. In formulating rations, adequate consideration should be given to the efficiency of conversion of feed to the end-product, be it weight gain or milk.

Table 6.1 Daily nutrient requirements (g TDN & g DCP) of a lactating buffalo cow weighing 350 kg (for maintenance, growth, milk production and gestation)

Milk yield (l/d)	Milk with 4.0 % fat		Milk with 6.0 % fat		Milk with 8.0 % fat	
	TDN	DCP	TDN	DCP	TDN	DCP
1	4710	565	4800	580	4900	600
2	5050	625	5230	655	5430	690
3	5390	685	5660	730	5960	780
4	5730	745	6090	805	6490	870
5	6070	805	6520	880	7020	960
6	6410	865	6950	955	7550	1050
7	6750	925	7380	1030	8080	1140
8	7090	985	7810	1105	8610	1230
9	7430	1045	8240	1180	9140	1320
10	7770	1105	8670	1255	9670	1410

Source: Ibrahim M.N.M. (1988)

6.7 Feeding of animals in different physiological states

6.7.1. Feeding guidelines for a newborn calf.

The dam's milk provides all the nutrients necessary for the new born calf. The fore-milk or colostrum contains in addition to nutrients, antibodies that provide protection against disease causing organisms. The value of colostrum is discussed in Chapter 28.

6.7.2 Feeding guidelines for growing animal

The guidelines for feeding calves and heifers up to maturity are detailed in Table 6.3, where for simplicity, the rations provided are based on grass and concentrates. Nevertheless in reality, the options that the farmer has, in choosing from among the various feed resources that are available at farm level, may be more extensive.

Table 6.2 Nutritive value of selected feeds (g/kg/fresh weight)

Feedstuff			DM	TDN	DCP
Grasses	Guinea -	Before flowering	210	116	19
		After flowering	301	105	09
	Napier grass		156	81	20
	Signal grass -	Before flowering	195	113	18
		After flowering	228	114	11
	Natural grass -	From road side	250	100	25
		From paddy field	180	88	18
Legumes	Gliricidia		226	142	50
	Ipil ipil		227	154	50
	Erythrina		237	119	64
	Wild sunflower		153	81	30
	Albizia		353	124	56
Tree leaves and shrubs	Jak leaves		314	151	28
	Girapala		250	165	31
	Madu-wal		300	146	25
Straw	Rice straw		900	300	0
	Rice stubble		850	315	0
	Ground nut straw		900	531	54
	Maize stover		903	447	29
Concentrates	Coconut poonac		923	683	148
	Rice bran		902	343	72
	Dairy feed mixture		940	620	124
	UMMB		900	764	46
	Rice polish		894	733	72

Source: Ibrahim, M.N.M. (1988b)

Table 6.3 Examples of practical rations for growing animals

Milk (litres)	Hay	Concentrate
Feeding of calf from day 04 to 4 weeks		
2.0 - 2.5	Small amount of hay or good quality fresh grass	Small amount of calf feed or prepared concentrate feed
Feeding of calf from 1 month to 2 months		
1.5 - 2.0	250 g of hay or 500 g fresh grass + 250 g legume leaves	200 g of calf feed or prepared concentrate feed

Feeding of calf from 2 months to end of 3 months		
0.50 - 0.75	750 g of hay or 1.25 kg fresh grass + 600 g legume leaves	350 g of a calf feed or prepared concentrate mixture
Feeding of calf from 4 months to end of 6 months		
	6 kg of fresh grass	1 kg of a cattle feed mix + 10 g of mineral mixture
or	6 kg of fresh grass + 4 kg of legume leaves	0.5 kg of concentrate mix + 10 g of mineral mixture
Feeding from 7 months to end of 9 months		
	11 kg of fresh grass	1 kg of a cattle feed mix + 10 g of mineral mixture
or	10 kg of fresh grass + 6 kg of legume leaves	0.5 kg of concentrate mix + 10 g of mineral mixture
Feeding from 10 months to end of 12 months		
	16 kg of fresh grass	1 kg of a cattle feed mix + 10 g of mineral mixture
Feeding from 13 months to end of 15 months		
	18 kg of fresh grass	1 kg of a cattle feed mix + 30 g of mineral mixture
or	15 kg of fresh grass + 9 kg of legume leaves	0.5 kg of concentrate mix + 30 g of mineral mixture
Feeding from 15 months to end of 18 months		
	21 kg of fresh grass	1 kg of a cattle feed mix + 30 g of mineral mixture
or	18 kg of fresh grass + 11 kg of legume leaves	0.5 kg of concentrate mix + 30 g of mineral mixture
Feeding from 19 months to end of 21 months		
	25 kg of fresh grass	1 kg of a cattle feed mix + 30 g of mineral mixture
or	22 kg of fresh grass + 12 kg legume leaves	0.5 kg of concentrate mix + 30 g of mineral mixture
Feeding from 22 months to end of 24 months		
	30 kg of fresh grass	1 kg of a cattle feed mix + 30 g of mineral mixture
or	25 kg of fresh grass + 15 kg legume leaves	0.5 kg of concentrate mix + 30 g of mineral mixture
Feeding from 25 months to end of 30 months		
	35 kg of fresh grass	1 kg of a cattle feed mix + 30 g of mineral mixture
or	28 kg of fresh grass + 16 kg legume leaves	0.5 kg of concentrate mix + 30 g of mineral mixture

6.7.2 Feeding guidelines for lactating animals (Feed formulation)

In formulating rations for lactating cows, the nutrient requirements for maintenance (which depends on body weight) and milk, both in terms of the yield and milk fat content have to be taken into consideration. Further a knowledge of the nutritive value of feedstuffs that are available to the farmer is necessary. The objective in feed

formulation is to match the requirements of the animal with the nutrient content of the formulated ration, keeping in mind the cost of the feed. The information provided in Tables 6.1 and 6.2 will be useful for this purpose. The method of calculation and the steps involved in the formulation of a feed ration is shown in Boxes 6.1 and 6.2.

As most feeding tables are given for a standard body weight and fat content, the values of g TDN and g DCP obtained from such tables must be corrected or adjusted for the body weight and the fat content of the cow under consideration. Standards for these adjustments are given in Box 6.1.

Box 6.1 Adjustment factors for different body weights and fat contents.

(a) *Adjustment for different body weights (Cattle and buffalo):*
For every 50 kg difference in body weight, add or subtract 300 g TDN and 25 g DCP

(b) *Adjustment for fat content in buffalo milk:*
For every 1% increase in fat content provide an additional 50 g TDN and 8 g DCP for every litre of milk produced.

(c) *As the milk from neat cattle is low in fat, adjustment for fat % in milk is not necessary.*

Box 6.2 Calculation of a ration for a lactating cow

Assume that a pregnant buffalo cow weighing 400 kg produces 5 litres of milk per day with 7 % fat. If the farmer has access to Guinea grass (1 month old), gliricidia, coconut poonac and urea molasses lick block, prepare a suitable ration to meet the daily nutrient requirements of the animal following the steps given below.

Step 1 Determine the TDN and DCP requirements of the cow from the information given in Table 6.1 and Box 6.1.

A 350 kg cow producing 5 L of milk with 6% fat requires 6520 g TDN and 880 g DCP (from Table 6.1). When adjusted for the weight (+50 kg) and fat content (+1% fat), the additional TDN and DCP requirements are + 300 and +25, respectively. Therefore,

Total requirement for 400 kg cow producing 5 L milk and 7% fat are 7070 g TDN and 945 g DCP

Step 2 Determine the nutritive value of the available feeds from Table 6.1.

	g. TDN	g. DCP
Guinea grass	116	19
Gliricidia	142	50
Coconut poonac	683	148
Urea molasses multinutrient mixture	764	46

Step 3 Ration formulation

Now select the appropriate combination of feed materials to match the nutrient requirements of the animal as given above. In feeding of ruminants, there is a limit to the intake of roughage feeds. The maximum intake that can be expected of a cow weighing 400 kg is 12 kg of dry matter (at 3% intake on DM basis) or about 60 kg fresh grass, if we assume that the dry matter content of grass is 20% and the cow has the ability to consume fresh grass at a maximum of 15% body weight per day.

Now calculate how much a cow weighing 400 kg and producing 5 L of milk containing 7% fat would receive, if she consumes good quality grass (Guinea grass, before flowering, with 116 g TDN and 19 g DCP) at maximum level of intake (3% body weight on dry matter basis). This will amount to 6960 g TDN (60 x 116) and 1140 g DCP (60 x 19). At this level of consumption, the cow will receive the total TDN and DCP requirements. Unfortunately, under normal conditions, cows neither receive good quality grass nor reach the optimum level of intake. Moreover, when poor quality roughage is fed the intake is low, because of low palatability and digestibility. Thus, as discussed elsewhere (Chapter 1) supplementation with concentrates (e.g. coconut poonac, rice polish), tree fodder (Gliricidia, Ipil-ipil) or urca-molasses-multinutrient feed mixtures, separately or in combination is necessary. In such circumstances the feed ration has to be balanced with supplements in a manner that will make it cost effective. One such example is given below.

Assume that the ration is made up of 50 kg of young Guinea grass (before flowering) and 3 kg of Glyricidia, the total TDN and DCP that could be obtained is given below.

	Dry matter Intake (kg)	TDN (g)	DCP (g)
(i) 50 kg Guinea grass grazed or cut and fed, will provide	10.5	5800	950
(ii) 3 kg Gliricidia will provide	0.7	426	150
(iii) **Nutrients supplied by grass and Gliricidia**	**11.2**	**6226**	**1100**

From the above calculation it is clear that there is a deficiency of TDN in the above ration. Therefore a third feed resource has to be included in the ration to overcome the TDN deficiency of 844 g (7070-6226). Because of the space limitation in the rumen, this has to be a concentrate like coconut poonac or urea molasses multinutrient mixture (UMM). So the farmer has two options.

(i) supplement with coconut poonac or
(ii) supplement with UMM.

Step 4:

If option 1 is followed one Kg of coconut poonac will provide 683 g TDN and 148g DCP. Accordingly, 1.24 Kg of coconut poonac is required to match 844 g of TDN (884/683). At the prevailing market price (e.g. mid 1997 prices - Rs.14/Kg), this supplementation will cost Rs.17.36. If however option 2 is followed, 1 Kg of UMM will provide 764 g of TDN accordingly 1.10 Kg of UMM is required to provide 844 g of TDN. This supplementation will cost only about Rs.7.04 (at the rate of Rs.32 per 5 Kgs UMM block).

Note that there is a Rs. 10 difference between options (a) and (b) above.

Note that the farmer has a choice between the following two rations:

	Ration A (kg)	**Ration B** (kg)
Guinea grass	50	50
Gliricidia	3	3
Coconut poonac	1.24	-
UMM Mixture	-	1.1

The ultimate choice will obviously be based on economic considerations, which in this case is ration B, which includes UMM.. One should also remember that UMM contains mineral elements such as calcium and phosphorus which are equally important nutrients as TDN and DCP.

Selected references

Abeygunawardena, H., Siriwardene, J. A. de S. and Subasinghe, D.H.A. (1998) Cattle and Buffalo Farming. *A training manual for extension workers.* National Science Foundation Press, Colombo, Sri Lanka.

Ibrahim, M.N.M. (1988a) *Feeding tables for ruminants in Sri Lanka.* Published under the Feed Advisory Act of Sri Lanka, Department of Animal Production and Health, Peradeniya, Sri Lanka.

Ibrahim, M.N.M. (1988b) *Feeding tables. A practical guide.* Department of Animal Production and Health, Peradeniya, Sri Lanka.

Subasinghe, D.H.A., Horadagoda, N.U., Abeygunawardena, H. and Siriwardene, J.A. de S. (1998) *Water buffalo - Improved utilization through new technologies.* National Science Foundation Press, Colombo, Sri Lanka.

CHAPTER 7

Endocrine Physiology of Reproduction

H. Abeygunawardena and I. S. Abeygunawardena

Introduction

A thorough understanding of endocrine physiology is a pre-requisite for management of fertility of cattle and buffaloes. Therefore, the aim of this introductory chapter is to review the basic concepts of reproductive physiology and physiological mechanisms controlling the sexual cycle of mammalian species.

The driving force of the endocrine system originates in the central nervous system. The specific area within the CNS which controls the reproductive system, is primarily located in the hypothalamus. The neurohormonal signal from the hyphothalamus controls the synthesis and release of trophic hormones from the pituitary gland. The trophic hormones from the pituitary gland in turn act on target tissues, particularly on gonads to produce hormones that exert a wide spectrum of actions on reproductive tissue as well as on various other body tissues. The gonadal hormones in turn control the functions of the hypothalamus and pituitary through a feedback mechanism. This loop is called hypothalamo-pituitary-gonadal system (Fig 7.1).

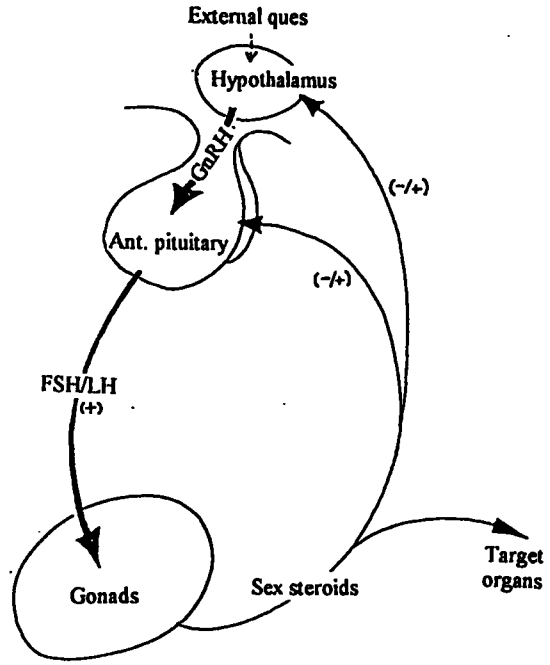

Fig. 7.1 Schematic presentation of hypothalamo-pituitary-gonadal system

7.1 Hypothalamus

The hypothalamus is the centre of neuro-endocrine, autonomic and homeostatic regulation. The hypothalamus is extensively interconnected and controlled by other

areas of the central nervous system through various neurotransmitter systems (Fig. 7.2).

According to the accepted model, hypothalamic neurons serve as neuro-endocrine transducers. They perform two functions. They act as (a) nerve cells that receive and transmit electrical information and (b) endocrine cells that release their secretory products into the blood stream. In other words, these cells convert neural information into hormonal signals. In these cells, neurohormones or precursor peptides are synthesised in the cell body and packed in secretory vesicles that are transported rapidly down the axon terminals where they are stored for release following stimulation. Using various immuno-cytochemical techniques, several types of hypothalamic neuronal systems have been described. Of the many neuronal systems, the GnRH-producing neuronal system is directly responsible for driving the reproductive system.

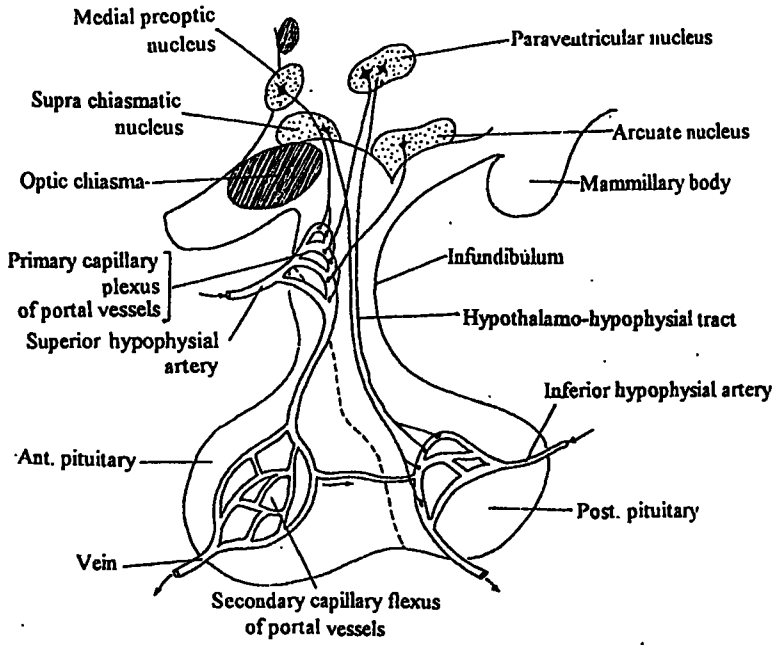

Fig. 7.2 Diagram showing the anatomical organisation of the hypothalamus and pituitary with their boundaries, main neural tracts and blood supply. (Redrawn from Johnson and Everitt, 1988)

The activity of the GnRH producing neurons are controlled by two control mechanisms: (a) neural control *via* neurotransmitter systems (e.g. adrenergic or dopaminergic neurotransmitter system) and (b) feed-back control by target gland hormones (e.g. oestrogens, progesterones). Neural control is very sensitive to external environmental cues such as daylight, temperature, humidity, etc., and it is through this component that environmental factors influence reproductive function.

7.2 Pituitary gland

The fully developed pituitary gland is an amalgam of hormone producing glandular cells (adenohypophysis = anterior pituitary) and neural cells with secretory functions (neurohypophysis = posterior pituitary). The anatomical organisation and vascular supply are depicted in Fig. 7.2.

The anterior pituitary gland (adenohypophysis) consists of five endocrine cell types: gonadotrophs, corticotrophs, somatotrophs, lactotrophs and thyrotrophs. Gonadotrophs have been shown to produce both LH and FSH. Posterior pituitary gland (neurohypophysis) is a continuation of hypothalamic neurons and secrete oxytocin and arginine-vasopressin of which the latter was formerly referred as antidiuretic hormone (ADH).

7.2.1 Relationship between GnRH and LH secretion

It is now well established that GnRH causes the synthesis and release of LH and FSH from the gonadotrophs. GnRH has been shown to be released in a pulsatile manner, with frequency and amplitude varying with the physiological stage of the sexual cycle. The anatomical substrate that is responsible for this pulsatile release of GnRH is not well elucidated as yet. A model based on the limited information, designate the anatomical substrate responsible for this pulsatile release of GnRH as the *GnRH pulse generator* (Fig. 7.3).

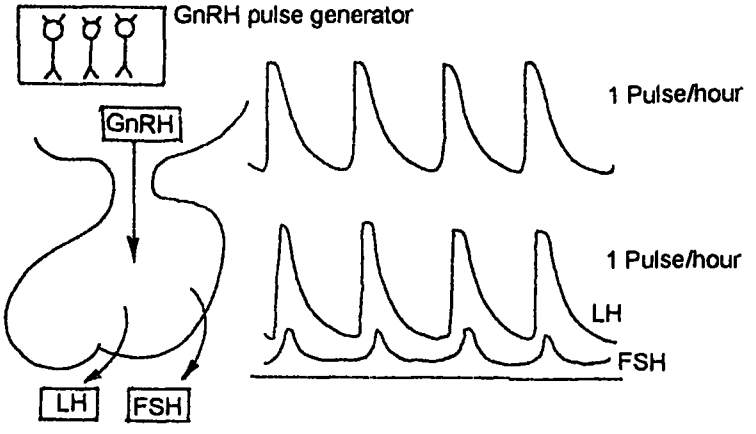

Fig 7.3 Schematic representation of GnRH neural pulsator ("pulse generator") and the relationship between GnRH pulses and LH and FSH episodes.

In this model, each GnRH pulse is expected to be followed by a LH pulse, but the 1 : 1 relationship is not always found as the translation GnRH signal is modified at the level of the pituitary. Further, the pulse frequency and amplitude have been shown to be entrained by the endocrine milieu and by the environmental, nutritional and stress factors. The firing frequency of GnRH producing neurons is determined by the stimulatory and inhibitory neural inputs by the different neurotransmitter systems such as the (i) adrenergic neuron system (predominantly stimulatory effect), (ii)

dopaminergic neuron system (both stimulatory and inhibitory effects), (iii) seretonergic neuron system (inhibitory effect) and (iv) opioid neuron system (predominantly inhibitory and minor stimulatory effect). The gonadal steroids act either *via* these neuro-transmitter systems or directly at the GnRH-producing neurons and bring about both the feedback inhibitory and stimulatory effects.

7.2.2 Kinetics of GnRH action

The kinetics of GnRH action at the level of gonadotrophs have been studied in great detail both *in-vitro* and *in-vivo* systems. It has been shown that the equilibrium between synthesis, storage, release and degradation of LH, shifts characteristically during the oestrous cycle. *In-vitro* studies have also shown that the half maximal dose required for the synthesis and release of LH (HMDs+r) is greater than the half maximal dose required for synthesis and storage (HMDs+s). This suggests that at a low level of GnRH pulsations (low frequency and/or low amplitude), LH is synthesised and preferentially stored over for release. This has been well demonstrated in the rat where the LH content of the pituitary increases gradually during the time interval between oestrus to proestrus, while the level in serum remains constant. This phenomenon has also been demonstrated in sheep and it is very likely that similar phenomena may occur in other species too. This may be the reason for the build up of LH stores in the pituitary during the luteal phase of the cycle, for surge release during the preovulatory period. This phenomenon has been used in the induction of oestrus with the use of progesterone-releasing devices in farm animal species.

7.2.3 Pattern of gonadotrophin secretion

In the female, both LH and FSH secretion show two distinct secretory patterns. This is primarily controlled by the GnRH secretory pattern and the changes in the responsiveness of the pituitary gland to GnRH:Tonic (basal), episodic (pulsatile) release with characteristic frequency and amplitude depending on the stage of the oestrous cycle and phasic surge release of LH and to an extent of FSH, which is superimposed on the basal secretory pattern. The basal secretory pattern of secretion promotes the development of both germinal and endocrine components of the ovary (pre-antral to antral follicle and steroidogenesis) and testes (spermatogenesis and steroidogenesis). The cyclic release occurs only in cyclic females. This phasic or surge secretory pattern promotes the final maturation of follicles (antral to preovulatory), oestrogen synthesis, ovulation and luteinization of the ovulated follicle.

7.2.4 Regulation of gonadotrophin secretion in the female

During the oestrous cycle in domestic animals and the menstrual cycle of the primates, the gonadal steroids and proteins very precisely regulate the pattern of gonadotrophin secretion dictated by the neural inputs *via* GnRH from the hypothalamus. Gonadal steroids exert two types of feedback effects and they appear to act both at the hypothalamic and pituitary level, but the relative importance of the sites vary with the species.

7.2.4.1 *Negative feedback effect:* Oestrogen at low circulatory levels feed-backly inhibits the gonadotropin secretion. This affect is very rapid in onset and detectable within 1 hour. Similarly, progesterone at high circulating levels feed-backly inhibits gonadotropin secretion. Further, at high concentrations (3-5 ng/ml), progesterone also blocks the positive feedback affect to oestrogen. In addition to these two steroids, ovarian inhibin, a protein secreted by developing follicles has been shown to inhibit FSH secretion selectively.

7.2.4.2 *Positive feedback effect:* Increasing circulatory levels of oestrogen (>200-400% increase from the basal level) stimulates the secretion of LH (and to a extent of FSH), resulting in a surge release of LH. This effect, in contrast to the negative effect is slow in onset and requires about 24-48 hours to manifest.

7.2.4.3 *Sites of negative and positive feedback effects:* There are two possible sites, namely the pituitary and the hypothalamus. At the pituitary level circulating oestrogens and progesterone (priming effects) could modulate the response to GnRH. At the hypothalamus, gonadal steroids could affect the GnRH producing neurons directly or indirectly (*via* neurotransmitter systems) and modulate the GnRH pulse frequency and amplitude. In some animal species, the pituitary appears to play a major role while in others, the hypothalamus seems to be more involved than the pituitary.

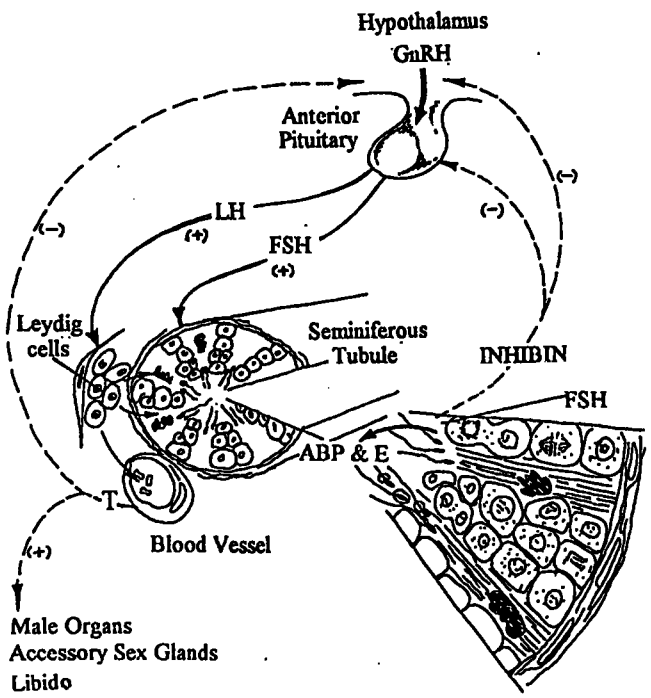

Fig. 7.4 Schematic diagram depicting the control mechanism of gonadotrophin secretion in the male. (Redrawn from McDonald and Pineda, 1989)

7.2.5 Regulation of gonadotrophin secretion in the male

The neuroendocrine mechanism governing the gonadotrophin secretion is basically similar to that in the female. The evidence suggests that two secretory products of the testes, namely testosterone and inhibin are involved in the feedback control of gonadotrophin secretion (Fig. 7.4)

7.2.5.1 *Testosterone:* Testosterone from the Leydig cells appears to inhibit LH secretion by reducing the LH pulse frequency with little affect on pulse amplitude. The pulse frequency of LH is primarily governed by the GnRH pulse frequency from the hypothalamus. Therefore, testosterone seems to act primarily *via* the hypothalamus.

7.2.5.2 *Inhibin:* Inhibin produced by Sertoli cells appears to exert feedback inhibition selectively on FSH secretion.

7.3 Gonads

7.3.1 Female gonads

The ovary contains oogonia and the transformation of oogonia into oocytes is referred to as oogenesis. Oogenesis commences during the embryonic stage. At the completion of oogenesis, a single layer of follicular cells surrounds the oocyst and it is called the primordial follicle. At birth, females of all domestic species carry a full complement of primordial follicles (for example approximately 100,000 - 150,000 primordial follicles per ovary). They are not replenished but decrease in number during the life of the animal. Folliculogenesis is defined as the formation of mature follicle(s) from a pool of primordial follicles. This process is diagrammatically represented in Fig. 7.5.

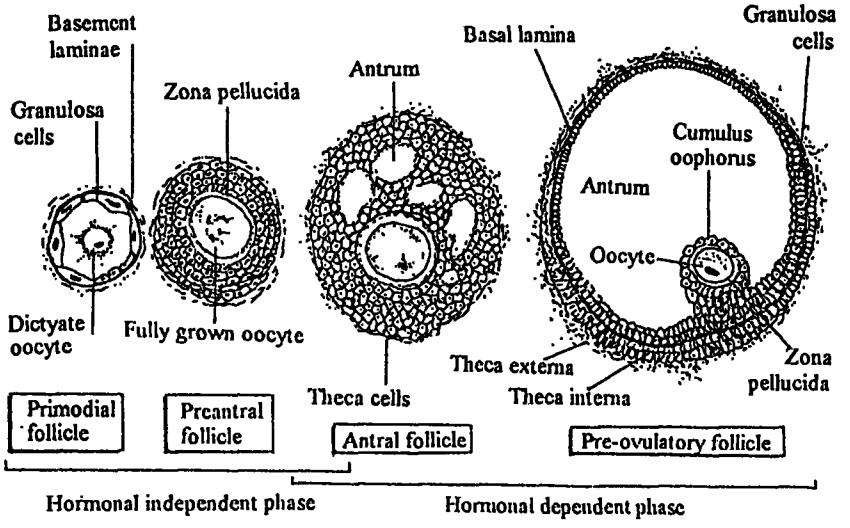

Fig. 7.5 Diagrammatic representation of different stages of follicular development. (a) primordial follicle, (b) pre-antral follicle, (c) antral follicle and (d) pre-ovulatory follicle. (Redrawn from Berne and Levy, 1983)

There are three phases of follicular development: (a) pre-antral phase, (b) antral phase and (c) pre-ovulatory phase. Follicular development is well controlled by pituitary and gonadal hormones.

7.3.1.1 *Folliculogenesis and endocrine control:* The initiation of growth of primordial follicles and progress through the pre-antral phase of follicular development occur independent of any pituitary hormonal control. However, towards the end of this phase of growth, a critical event in the life of the follicle occurs. In the early developing follicle, the receptors for LH are primarily located in the thecal cells while the receptors for FSH are found predominantly in granulosal cells. The thecal cells in response to LH produce aromatizable androgens, the substrate for oestrogen synthesis in granulosa cells, where aromatase enzyme production is stimulated by FSH. Oestrogen in cooperation with FSH stimulates the development of binding sites for LH on granulosa cells which hitherto lacked them. The acquisition of LH receptors on granulosa cells determines whether the follicle enters into the second phase of follicular development and henceforth the follicle becomes critically dependent upon pituitary gonadotrophin stimulation. Many of the pre-antral follicles do not go beyond this stage and hence undergo degeneration or *atresia.* In the presence of tonic levels of FSH and LH in the circulation, the follicles continuously develop. In this process, granulosa cells and thecal cells continue to proliferate resulting in a further increase in follicular size. During this phase, there is a steady increase in the synthesis of androgens and oestrogens within the follicle. Androstenedione and testosterone are the principle androgens while oestradiol 17β is the principle oestrogen. Oestrone is also secreted in most species. The production of steroids is under the influence of gonadotrophins and the synthesis of oestrogen catalyses further follicular growth at this stage. Hence, the process of follicular development involves the cooperation of two cell types (granulosa cells and thecal cells) and two gonadotrophins (FSH and LH). The model proposed to explain this cooperation is called *two-cell-two-gonadotrophin hypothesis* (Fig. 7.6).

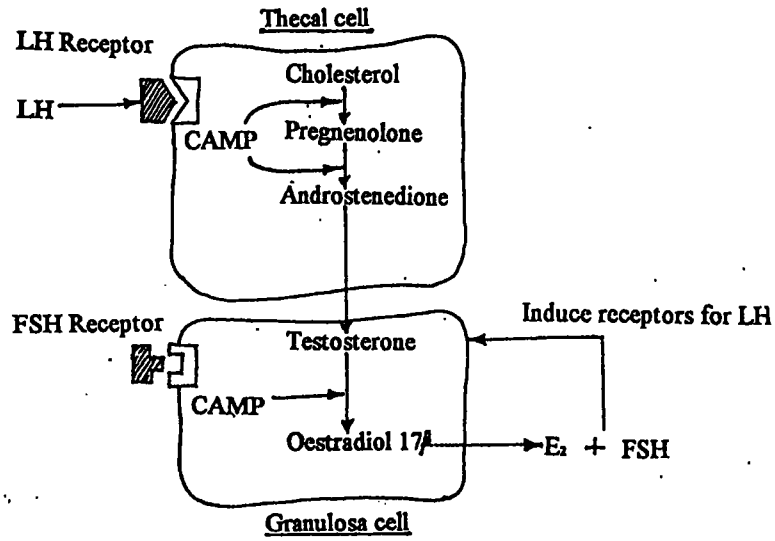

Fig.7.6 Diagrammatic representation of two-cell-two-gonadotrophin hypothesis.

The terminal development of the antral follicle or third stage of follicular development is LH surge dependent. At this stage of development, the granulosa cells possess LH receptors and hence LH can bind to both granulosa cells and thecal cells as well. The affect of the LH surge on these advanced follicles is two fold: firstly, it causes the terminal growth changes in follicle cells and in the oocyte, which culminates in oocyte expulsion from the follicle (ovulation); secondly, it changes the whole endocrinology of the follicular cells. The cells that hitherto secreted oestrogen, now become the cells of the corpus luteum following ovulation, and begin to secrete progesterone.

7.3.1.2 *Ovulation:* During the third phase of follicular development considerable increase in follicular size occurs, the increasing size of the follicle and its position in the cortex of the ovarian stroma cause the follicle to bulge out from the ovarian surface leaving only a thin layer of epithelial cells separating the follicular wall from the peritoneal cavity. The follicle then ruptures at this point (which is called the stigma) causing the fluid to flow out to the surface of the ovary carrying with it the oocyte and surrounding granulosa cells (cumulus oophorus and corona radiata cell mass). The mechanism of ovulation is not well understood yet, but the model, based on available information is depicted in Fig. 7.7.

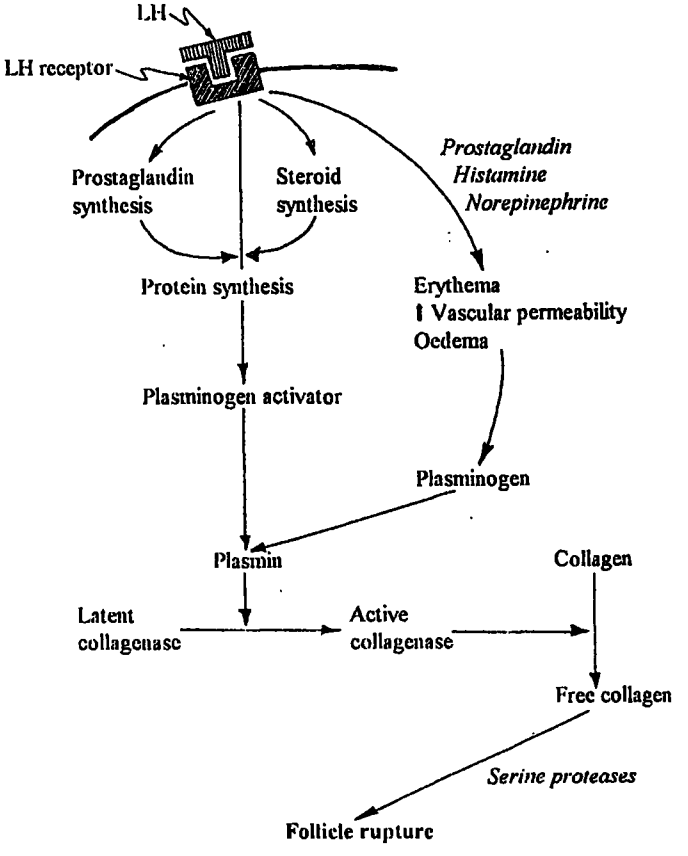

Fig. 7.7 Multifactor hypothesis to explain mechanism of ovulation. (Adapted from Tepperman and Tepperman, 1987).

According to this model, ovulation is initiated by the LH surge through production of steriods, prostaglandins and a battery of specific proteins. One of the specific proteins produced is plasminogen activator which converts the plasminogen in the follicular fluid and oedema fluid into plasmin. Plasmin then acts on the latent collagen which are attached to collagen fibers. This leads to proteolysis of collagen fibers, resulting in a decrease in the tensile strength of the follicle wall. Follicle ruptures at the existing intrafollicular pressure at the weakest point.

The residual expulsion of the parts of the ovulated follicle within the ovary then collapse into the space left by the fluid, the oocyte and the cumulus cell mass. This is followed by the formation of a blood clot within this cavity. The collapsed follicle therefore comprises a fibrous core, surrounded by several layers of collapsed granulosa cells, enclosed within a fibrous outer thecal capsule.

7.3.1.3 *Luteinization:* The collapsed follicle following ovulation transforms into a corpus luteum. The morphological and functional changes of follicle cells from ovulation to corpus luteum formation is called luteinization. Both types of follicular cells, granulosa and thecal cells, are incorporated into the developing corpus luteum. The fibrous core undergoes fibrinolysis, and this process is associated with the breakdown of the membrane between theca and granulosa cell layers and vascularization of lutein cells. The lutein cells cease further division and undergo hypertrophy. The transformed cells now produce progesterone and this change in the steroidogenesis process is the result of loss of 17∝ hydroxylase activity. Endocrine support for luteal maintenance shows considerable species variation. Ovulation is caused by LH surge and it is now well established that the luteinization process is initiated with the LH surge. Further, the maintenance of the corpus luteum also requires continued support of pituitary LH (except in some species).

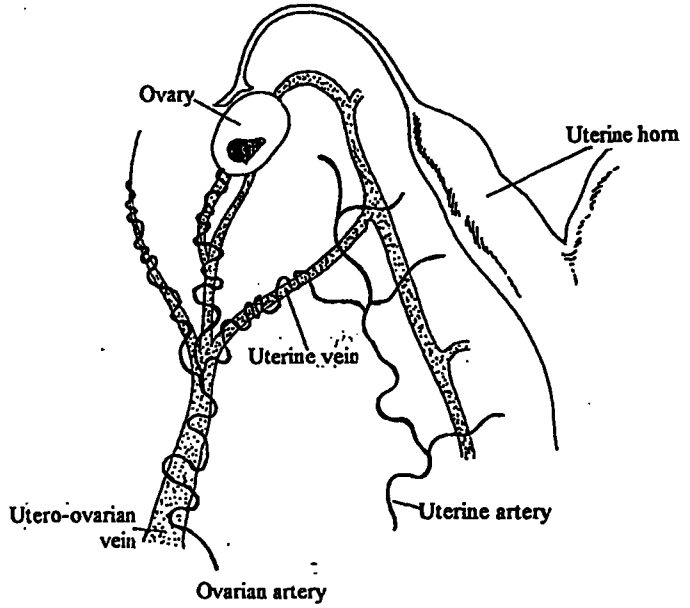

Fig. 7.8 Relationship between uterine $PGF_{2\alpha}$ and CL in the ovary. (Redrawn from Peters and Ball, 1995)

7.3.1.4 *Luteolysis:* Luteal regression or luteolysis involves the collapse of the lutein cells, ischaemia and progressive cell death with a consequent fall in the output of progesterone. It is now well established that the endometrium following a period of progesterone dominance produces a luteolytic substance that passes to the ovary bearing CL *via* a counter current mechanism. This substance has been identified as prostaglandin $F_2\alpha$, which is believed to be the luteolytic factor in the majority of domestic species (Fig. 7.8).

The more recent scientific evidence suggests that the CL of many domestic species produce protein hormones (oxytocin and relaxin) in addition to progesterone. The synthesis and release of these hormones are influenced by utero-ovarian factors. The evidence suggests that oestrogen from the developing follicle induces receptors for oxytocin in the endometrial cells. Oxytocin of ovarian origin stimulates the production of prostaglandin $F_{2\alpha}$ in the endometrial cells acting *via* the oxytocin receptors and this substance reaches the CL *via* the ovarian artery following transfer from the uterine vein through a counter current mechanism (Fig. 7.9).

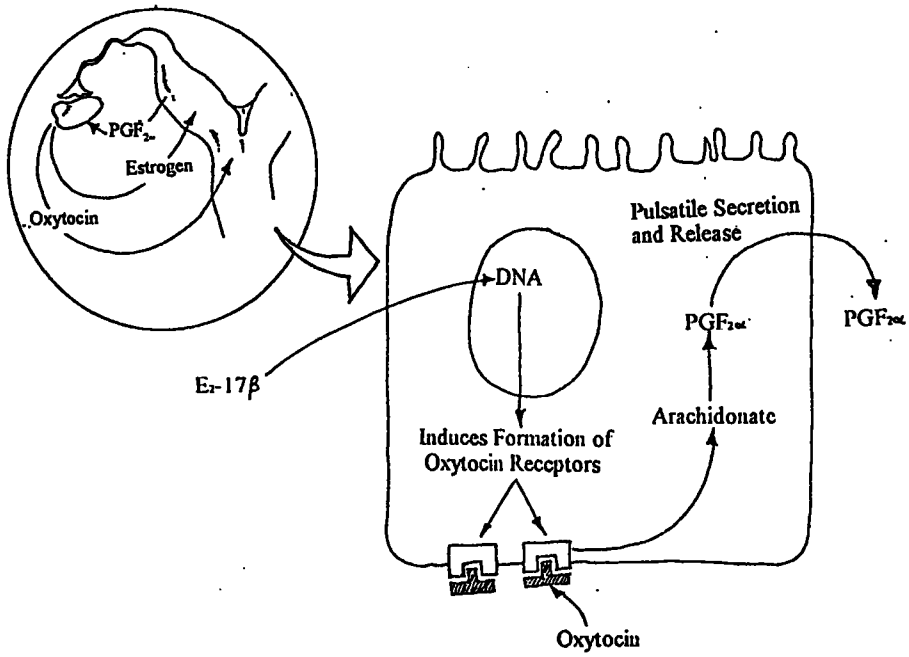

Fig. 7.9 Relationship between ovarian oestrogen, oxytocin and uterine prostaglandins in the mechanism of luteolysis. (a) Relationship between luteal oxytocin, follicular oestrogen and uterine progesterone $F_{2\alpha}$ (b) Oxytocin receptors regulation by follicular oestrogen and pulsatile secretion and release of $PGF_{2\alpha}$ from endometrial cells. (McDonald and Pineda, 1995)

7.3.2 Male gonads

In all domestic animal species, the testes are paired organs, which descend during foetal life from the abdominal cavity to lie in a pouch, the scrotum. Structurally, the testes consist of two functional compartments, seminiferous tubules and interstitial tissue (Fig. 7.10).

tissue (Fig. 7.10).

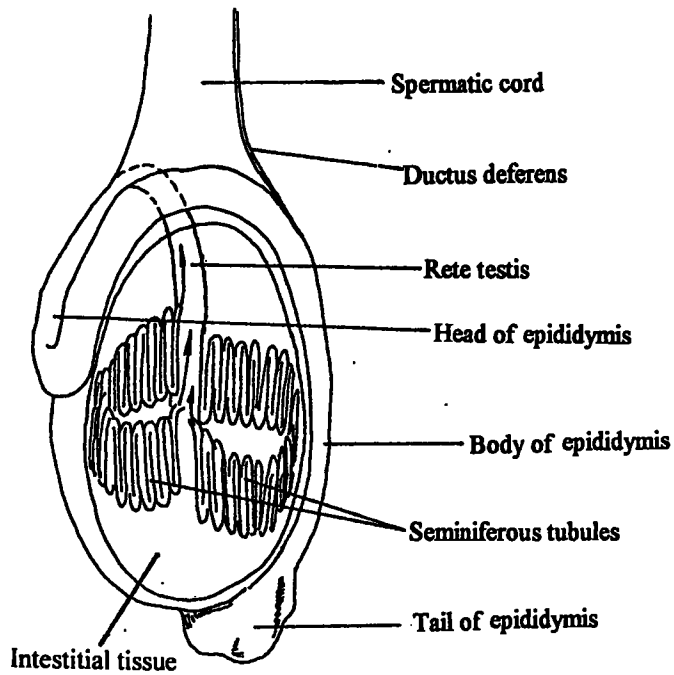

Fig. 7.10 Structural organisation of mammalian testes. (Adapted from Setchel, 1978)

7.3.2.1 *Endocrinology of testis:* Testes are very active endocrine organs. The principle hormone is testicular androgens. Besides that the testes produce several other steroids, peptide hormones such as the Mullerian inhibiting factor and inhibin. Testosterone is the primary androgenic steroid that is produced, but besides this other androgens such as androstenedione (more in prepubertal animals), dihydrotestosterone and non-androgenic steroids, such as, dehydroepiandrosterone sulphate and oestrone sulphate have been shown to be secreted by the testes. The primary component of testes that is active in hormone production are the Leydig cells of interstitial tissues which function under the influence of luteinizing hormone. Besides that, particularly in young animals, Sertoli cells have been shown to convert androgens into oestrogens. But this function is not observed in adults and hence it has been widely accepted that Leydig cells are the main source of androgens and oestrogens in adult animals. Testosterone is a fat-soluble compound and hence can traverse cellular barriers separating major testicular compartments. Within the tubular compartment, androgen is bound to Sertoli cells and are also in equilibrium with androgen binding protein. Androgens have several affects on different body systems and at different stages of growth.

7.3.2.2 *Endocrine control of spermatogenesis:* Studies have clearly demonstrated that the pituitary through FSH and LH control testicular function. In these studies, it has been shown that FSH is specifically bound to Sertoli cells while the LH is specifically bound to Leydig cells. However, the roles of the two gonadotrophins in spermatogenesis and the precise mechanisms through which these hormones and

others control spermatogenesis has not been well deliniated. There appears to be species differences with regard to the importance of each hormone in maintaining testicular function. The information is very scanty too, particularly with regard to farm animal species.

The experimental findings have established that (a) spermatogenesis is a hormone dependent process and is endocrinologically controlled by pituitary hormones (LH, FSH and also prolactin) and (b) testosterone, the product of Leydig cells, is involved in the maintenance of spermatogenesis.

7.4 Biochemical characteristics and actions of GnRH, LH, FSH and related hormones

7.4.1 Gonadotrophin-releasing hormone (GnRH)

This is a 10 amino acid peptide with a molecular weight (MW) of approximately 1000 daltons. Its plasma half-life is less than 10 minutes, but its synthetic analogs have longer half-lives and hence analogs are used for therapeutic purposes. It stimulates the biosynthesis, glycosylation and the release of both FSH and LH in the gonadotrophs and also the release of these two hormones from gonadotrohps.

7.4.2 Luteinizing hormone (LH)

This is a glycoprotein hormone with a MW of approximately 30,000. Its plasma half-life is about 15 - 30 minutes.

(a) In the female this hormone:

(i) stimulates the further growth of the developing follicle towards maturity (pre-antral → antral to preovulatory).
(ii) stimulates the steroidogenesis and hence synthesis of oestrogens in developing follicles.
(iii) through a surge release causes ovulation of mature follicle and luteinization of the ovulated follicle.
(iv) stimulates steroidogenesis in the luteal tissue and hence is luteotrophic and maintains the corpus luteum.

(b) In the male, it:

(i) acts on the Leydig cells and causes androgen production
(ii) also appears to bind to Sertoli cells and is involved in the spermatogenesis process

7.4.3 Follicle stimulating hormone (FSH)

It is a glycoprotein with two sub-units with a MW of approximately 32,000. The plasma half-life of FSH is about 2-4 hours.

(a) In the female, this hormone it:

(i) causes multiple follicular growth without substantial oestrogen production and ovulation.
(ii) acts primarily through granulosa cells
(iii) in concert with oestrogen, it promotes the development of LH receptors on the granulosa cells

(b) In the male, it:

(i) stimulates spermatogenesis with androgen
(ii) stimulates the production of androgen-binding protein from Sertoli cells.
(iii) stimulates the production of inhibin by Sertoli cells which feed-backly suppress the FSH secretion selectively.

7.4.4 Prolactin

It is a protein hormone with a MW of approximately 20,000 and a plasma half-life of about 15 minutes.

(a) In the female, it:

(i) is luteotrophic in some species (canine and rodents)
(ii) stimulates lactogenesis and galactopoiesis along with corticosteroids, growth hormone and insulin in all mammals.
(iii) acts as an antigonadal hormone in pathological elevations, for example in lactational amenorrhoea in women.

(b) In the male, the physiological effects are less characterized compared to that of the female.

(i) It appears to act on testicular cells and brings inhibitory as well as stimulatory affects on steroidogenesis and spermatogenesis, depending on the species.
(ii) In pathological elevations in man, it is associated with impaired fertility, decreased circulatory levels of testosterone and loss of libido.

7.4.5 Human chorionic gonadotrophin (hCG)

This is a glycoprotein, produced by chorion of human pregnancy with a MW of approximately 37,000. Its plasma half-life is approximately 6 hours. It is used widely for therapeutic purposes and predominantly exerts LH-like activities in many species.

It's action is to:

(i) stimulate streoidogenesis in gonadal tissues

(ii) stimulate follicle maturation, ovulation and luteinization
(iii) maintain luteal function
(iv) induce ovulation

7.4.6 Pregnant mare serum gonadotrophin (PMSG)/Equine chorionic gonadotrophin

It is secreted by the cells of the endometrial cups in equine pregnancy. It is a glycoprotein with MW estimates ranging from 28,000 to 65,000. The plasma half-life ranges from 50 to 120 hours. It exerts predominantly FSH-like activities with minor LH-like activities in farm animals (except in the horse where it has more LH-like effects) and also induces follicular development.

7.5 Biochemical properties and physiological actions of gonadal sex steroids and prostaglandins

Both progesterone and oestrogens are synthesised *de novo* from acetate *via* cholesterol. The trophic hormones for gonadal steroidogenesis are FSH and LH. These hormones, stimulate the steroid synthesis by acting at the rate limiting steps of steroidogenesis. The major classes of steroid derivatives *via* pregnenolone from cholesterol are (1) progesterones, (2) androgens, (3) oestrogens and (4) corticosteroids.

7.5.1 Progesterone

Naturally occurring progesterones have different biological potencies.

Name	Relative potency
Progesterone (P)	100%
17∝ hydroxy progesterone (17-OHP)	40-70%
20∝ hydroxy progesterone (20-OHP)	5%

Its physiological actions are to:

(i) prepare the uterus to receive conceptus - hyperplasia and hypersecretion of endometrial glands.
(ii) maintain the uterus in quiescence during pregnancy.
(iii) stimulate growth of the mammary gland.
(iv) exert mild effects on Na^{2+} loss *via* distal convoluted tubules of kidney
(v) exert mild catabolic effects (but in pregnancy progesterone exerts anabolic effect - increase appetite and deposition of fat in fat stores).
(vi) exert negative feedback control of gonadotrophin secretion.

7.5.2 Oestrogens

Naturally occurring oestrogens have different biological potencies

Name	Relative potency
Oestradiol 17β - (oestradiol or E_2)	100%
Oestriol (E_3)	10%
Oestrone (E_1)	1%

Its physiological actions are to:

(i) stimulate secondary sex characters of the female.
(ii) prepare the uterus for spermatozoa transport (contraction of smooth muscles, hypersecretion of cervical and uterine glands)
(iii) increase the vascular permeability and tissue oedema
(iv) stimulate growth and activity of mammary gland and endometrium
(v) prepare the uterus for progesterone action (oestrogen primes the uterus by inducing receptors for progesterone)
(vi) exert mild anabolic effects and stimulate calcification.
(vii) exert feedback control on gonadotropin secretion, both positively and negatively.
(viii) induce sexual behaviour in some species
(ix) stimulate immune responses and body defence mechanisms.

7.5.3 Androgens

Naturally occurring androgens have different potencies

Name	Relative potency
5∝-dihydrotestesterone-(DHT)	100%
Testosterone (T)	50%
Androstenedione	8%
Dehydroepiandrosterone (DHA)	4%

Their physiological actions are to:

(i) induce and maintain sexual differentiation of primitive (undifferentiated) gonadal primordial and male somatic tissues
(ii) induce secondary sex characters of males (deep voice, body hair, penile growth, etc.) and body hair of females.
(iii) induce the differentiation of and maintenance of accessory sex organs

(iv) maintain spermatogenesis
(v) influence sexual and aggressive behaviour in males and females
(vi) promote protein anabolism, somatic growth and ossification
(vii) regulate secretion of gonadotrophin (testosterone only).

7.5.4 Prostaglandin $F_{2\alpha}$

It is a biologically active lipid, synthesized from arachidonic acid which is a polysaturated C20 fatty acid found abundantly in every tissue of the body.

Its actions are to:

(i) stimulate involution of Cl.
(ii) stimulate contraction of smooth muscles of the reproductive organs - uterus, oviduct, etc.
(iii) induce in ovulation
(iv) stimulate 'ripening' of cervix at parturition

Selected references

Berne, R.M. and Levy, M.N. (1983) *Physiology* C.V. Mosby Company, Toronto, Canada.

Johnson, H. and Everitt, J. (1989) *Essential Reproduction* (3rd edition), Blackwell Scientific Publications, Oxford, UK.

McDonald, L.E. and Pineda, M.H. (1989) *Veterinary Endocrinology and Reproduction* (1989) (4th edition). Lea and Febiger, Philadelphia, London.

Peters, A.L. and Ball, P.J.H. (1995) *Reproduction in Cattle* (2nd edition) Blackwell Science Ltd., London, UK.

Setchel, B.P. (1978) *The Mammalian Testes,* Cornell University Press, New York, USA.

Tepperman, J. and Tepperman, H.M. (1987) *Metabolic and Endocrine Physiology.* (5th edition). Year Book, Medical Publishers Inc., Chicago & London.

CHAPTER 8

Sexual Cycle of Cattle and Buffaloes

H. Abeygunawardena and I. S. Abeygunawardena

Introduction

Sexual activity commences with onset of puberty and recurs in a cyclic manner throughout the lifespan of the animal.

8. 1 Puberty in cattle and buffalo

In the heifer, puberty is defined as the time at which the first oestrus accompanied by spontaneous ovulation occurs. In the male, puberty is defined as the time at which the ejaculate contains sufficient numbers of mature spermatozoa to impregnate a female. In cattle by convention this has been specified as the first ejaculate which contains more than 50 million spermatozoa with at least 10% progressive motility. The process of puberty is influenced by many factors, genotype, nutrition and accompanying growth rates are the primary factors.

8.1.1 Factors affecting puberty

The process of puberty appears to be affected by a wide variety of factors, both intrinsic and extrinsic to the animal.

8.1.1.1 *Intrinsic factors*

(a) *Genetic make up*: The genetic make up of the animal affects the pubertal process. For example, temperate breeds of cattle, sheep and goat attain puberty earlier than their tropical counterparts. Similarly, small breeds of many animal species attain puberty earlier than the larger breeds. e.g. Jerseys attain puberty by 8-10 months of age, compared to Holsteins that attain puberty by 11-13 months. Smaller canine breeds attain puberty (5-8 month) earlier than larger breeds (8-10 month).

(b) *Sex*: The sex of the animal also affects the pubertal process. In many animal species, females reach puberty earlier than males. This is particularly true in humans. But in animals, the pubertal process of the female is more sensitive to environmental factors and hence, they may take longer than the male under adverse environmental conditions.

8.1.1.2 *Extrinsic factors*

(a) *Photoperiod*: It has been shown that puberty in man occurs earlier in the tropics than in temperature zones and this may be explained by the fact that tropical man is exposed to longer day lengths than the temperate

man. In western societies, there has been a gradual decrease in the age at puberty over the last few decades. In 1940, the average age at puberty was 17 years and today it is around 13.5 years. Apart from improved socio-economic conditions, the exposure of man to light over a long photoperiod (with the advent of electricity) may have been a contributory factor. In seasonal breeders, such as sheep, early puberty is attained in lambs that have sufficiently developed the hypothalamic-pituitary axis, when exposed to decreasing photoperiods. Hence, lambs born in early spring attain puberty at an earlier age (150 days) than lambs born in late spring (400-500 days).

(b) *Nutrition*: In general a high plane of nutrition favours attainment of earlier puberty, while a low plane of nutrition delays puberty. Temperate breeds of cattle in the topics that were not provided improved nutrition showed delayed puberty. It is well known that food intake, growth rate and body weight are highly correlated with the onset of puberty and subsequent reproductive functions.

In many animal species, it has been shown that there is a critical body weight that must be attained to trigger the onset of puberty. In the human in western societies, it has been shown that, although the age at puberty has changed considerably during the past few decades, the body weight at menarche has remained constant at about 45-47 kg. In cattle, it has been suggested, that animals should attain 2/3 of the mature body weight to trigger the onset of puberty.

8.1.1.3 *Other factors:* Many other factors have been identified as having effects on the pubertal process, for example, interaction with the opposite sex favours early puberty, while stressful environmental factors such as high ambient temperature and humidity appear to delay puberty.

Some breeds have an inherent potential for higher birth weights and growth rates, when animals are provided adequate nutrition. Growth rates are also influenced by many factors such as pre-partum nutrition, disease and climatic conditions. It has been clearly demonstrated that continuous inbreeding without selection for higher birth weights and growth rates, and low levels of nutrient intake are associated with slow growth rates and delayed puberty, while a higher level of selection intensity and a high plane of prepubertal nutrition result in higher growth rates and early puberty.

8. 2 Seasonality of reproduction

The wild ancestors of the bovidae family were considered to be seasonal breeders, but in the process of domestication they have lost this seasonality with the provision of the conducive conditions for the survival of the species. In the temperate region, calving usually occurs in spring and summer when the environmental factors (temperature and food availability) are conducive for the survival of the young. The environmental determinant of this seasonality in temperate regions is the photoperiod; i.e. change of daylight period between seasons. During the course of domestication, in temperate regions, dairy and beef cattle have been selected against this seasonality with the

provision of a conducive environment. In the tropics, the situation is different. There is no great variation in day length or ambient temperature throughout the year. However, cattle and buffaloes in the tropics show a strong seasonality in calving and seasonal calving is closely linked to the seasonal rainfall pattern.

Work done in Sri Lanka, has demonstrated that the seasonality of calving is linked to the nutrient supply, where pasture production varies with rainfall. In most part of the country, particularly in the dry zone areas, the majority of calvings occur during the months from October to December. This period coincides with the north-east monsoonal rains (September to December) and is characterised by an abundance of pasture and fodder.

The analysis of conception dates with the environmental variables revealed that those animals that calve early in the calving season were exposed to very favourable conditions such as lush, green pasture and mild ambient temperature and hence these animals appear to resume post-partum ovarian activity earlier than those animals that calve late in the season. These late calvers, would then be exposed to high ambient temperature and scarcity of pasture and fodder and hence there is a delay in the onset of post-partum ovarian activity. This work as well as the work of others, clearly suggest that seasonality of calving which is seen both in cattle and buffaloes, primarily in the dry and intermediate zones, is due to entrainment of post-partum ovarian activity by nutrient supply which is linked to seasonal rainfall.

8.3 Oestrous cycle of cattle

As in other domestic mammals, cattle exhibit regular cyclic changes in reproductive organs and sexual behaviour. The sexual cycle is called the *oestrous cycle* and the duration of the oestrous cycle is approximately 21 days, with a range of 18 to 24 days. The oestrous cycle by convention is divided into 4 phases: *proestrus, oestrus, metoestrus and dioestrus*. But more correctly, it is now divided into two phases called the *follicular phase* and the *luteal phase*. The endocrine profile and phases of the oestrous cycle are depicted in Fig. 8.1 (a), and the temporal relationship between the LH surge, oestrus and ovulation are shown in Fig. 8.1 (b).

8.3.1 Luteal phase

- The luteal phase commences with ovulation and lasts for about 15 to 16 days, at which time regression of the corpus luteum (CL) commences.
- It is a progesterone dominant phase.
- Follicular growth occurs in two waves: the first wave commences early in the cycle (days 2-3), reaches a peak by mid-cycle (days 11-12) and subsequently follicles undergo atresia. The second wave, which produces the follicle that is destined to undergo ovulation commences by mid-cycle (days 11-12) and from this crop, one or two follicles gain dominance and eventually reach the preovulatory stage and undergo ovulation at the end of the follicular phase (note: some researchers describe 3 follicular waves during the oestrous cycle).

- Secretion of prostaglandin $F_{2\alpha}$ commences around day 16-17 of the cycle.

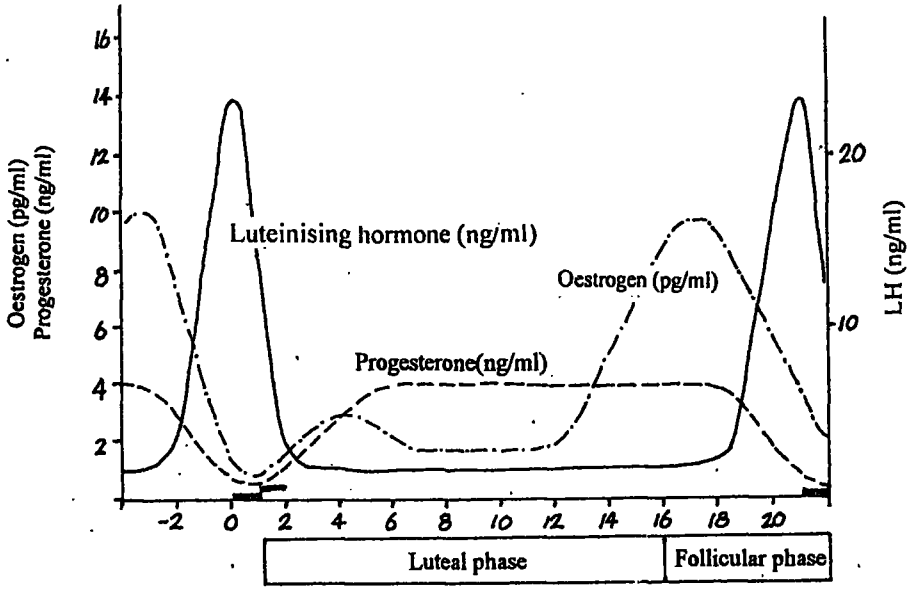

Fig. 8.1(a)

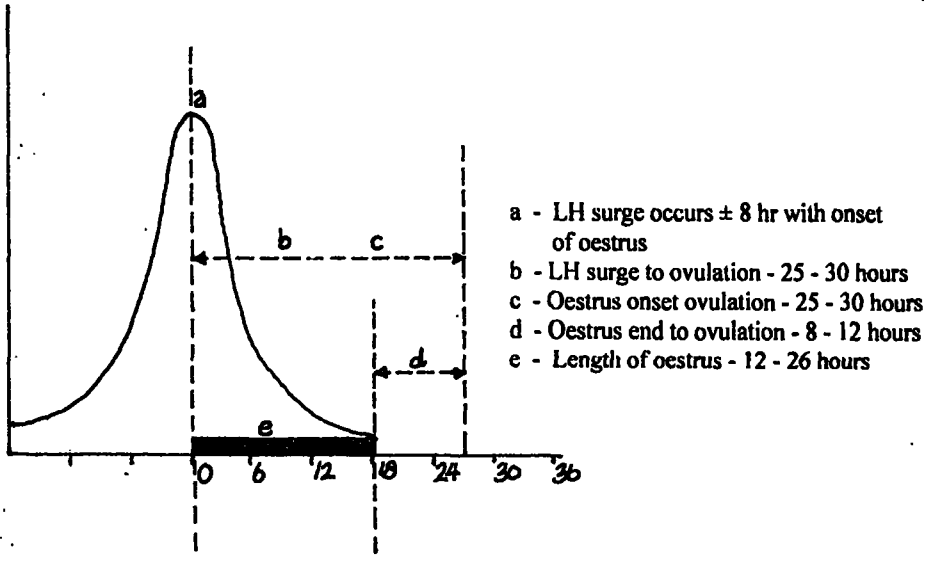

Fig. 8.1(b)

Fig. 8.1 (a) Phases and endocrine profile of oestrous cycle of cattle and (b) Temporal relationship between LH surge, oestrus and ovulation. (Adapted from McDoanald and Pineda, (1989)

8.3.2 Follicular phase

- The follicular phase is relatively of short duration (3 to 4 days) and commences with the onset of regression of the CL and ends with ovulation.
- This period is endocrinologically characterised by rising oestrogen from developing follicles, declining progesterone and rising levels of luteinizing hormone (LH) which culminate in the LH surge.
- Animal becomes sexually active during this period and within a narrow window of this phase, the animals become sexually receptive (accepts the male for coitus). This narrow window is called *oestrus.*

8.3.3 Oestrus

- It is the sexually receptive period
- This period begins with the acceptance of the male and on an average lasts for 18 hours within a range of 2 to 26 hours.
- Sexual receptivity and behavioural changes are mediated by rising levels of oestrogens in association with declining levels of progesterone, which act at the higher centres in the central nervous system as well as on other target organs such as the uterus, vagina, vulva, ligaments and muscles of perineum region.

8.3.4 Ovulation

- Ovulation refers to the release of the ovum from a mature follicle which occurs approximately about 25 to 30 hours following the LH surge.
- The occurrence of the LH surge very closely coincides with the onset of oestrus, within a range of $\pm$ 8 hours

8.3.5 Signs of oestrus

Characteristic signs of oestrus for descriptive purposes could be divided into two groups: behavioural and physiological signs.

8.3.5.1 *Behavioural signs*

- Cows in oestrus solicit other cows to mount them and may also mount on other animals.
- Cows in oestrus would stand for mounting by other cows or a bull.
- Cows becomes restless and depart from routine activities such as eating and drinking .
- Cows may sometimes become aggressive and bellow frequently.

8.3.5.2 *Physiological signs*

- Increase in mucous secretion in the cervix and vagina results in the discharge of clear, glassy strings of mucus from the vulva.

- Swelling and parting of vulval lips and relaxation of pelvic ligaments and perineal musculature
- Reddening of vulval and vestibular mucosa.
- Slight reduction of feed intake and milk yield.

8.3.5.3 *Other signs*

- Increase in frequency of urination
- Increase in electrical conductivity of vaginal mucus (due to increase in ionic content in mucus).
- Increase in ferning pattern of vaginal mucus (due to increase concentration of ions).

8.3.5.4 *Main criteria for detecting a cow in oestrus:* The readiness of a cow to stand to be mounted by other cows or a bull is the most reliable criterion. This would be evidenced by ruffled hair in the rump region and soiled flank.

8.4 Oestrous cycle of buffaloes

The oestrous cycle in buffaloes has been shown to be similar in many aspects to that of cattle. The average duration is approximately 21 days, with a range of 18 to 24 days. Endocrine studies have also shown similarities to that of cattle. The major differences, however are in the intensity of manifestation of behavioural and physiological oestrous signs. The intensity of signs in the buffalo is less than those in cattle. The variation in intensity and the frequency of occurrence is given in Table 8.1.

From the above data it is very clear that oestrous signs are not as consistent as in the case of cattle, and this makes oestrous detection in buffaloes somewhat more difficult than in cattle. The work done in Sri Lanka shows that oestrous signs are less pronounced in indigenous buffaloes and crosses than in pure river type buffaloes.

8. 5 Fertilisation and implantation

Fertilisation takes place at the ampulary region of the oviduct, and the zygote (fertilised ovum) will remain at the oviductal site of fertilisation for a further period of a few more hours and commence the downward migration towards the uterus. During the oviductal stay, the zygote undergoes cleavage (mitotic division) and goes through the morula and blastocyst stages. The bovine embryo at the 8-16 cell stage (morula stage) enters the uterus and this occurs between 72 and 96 hours after fertilisation. The free-floating embryo within the uterus undergoes further division and development and enters the blastocyst stage by 7 to 8 days post-fertilisation. There is an extensive migration within the uterine horn and the blastocyst hatches out from the *zona pellucida* by day 9 to 11. The blastocyst undergoes further elongation while floating within the uterus during next 10 to 12 days before commencement of adhesion and apposition to endometrium between day 22 and 28 post-fertilisation.

Table 8.1 The frequency of important oestrous signs in the river buffaloes.

Symptom	References					
	Gill *et al.* (1973) India (n=112)	Rao and Kodagali (1983) India		Singh *et al.*(1984) India (n=31)		Kazini(1981) Pakistan (n=31)
		Heifer (n=151)	Cows (n=49)			
Swelling	ns	ns		pronounced slight	18.5% 79.5%	96%
Free flowing of mucous discharge	17.0	78.5%	83.7%	copious little	46.7% 33.7%	48%
Hyperaemia of vulva/vagina	64.8%	83.4%	86.8%	pronounced slight	66.3% 33.7%	96%
Frequent urination	ns	51.7%	83.7%		34.8%	ns
Bellowing	25.2%	51.0%	68.8%	continuously seldom	58.7% 21.7%	8%
Uterine contraction	85.5%	53.0%	64.5%		ns	ns

ns = not studied

The implantation process is non-invasive and superficial. It goes through several phases of trophoblastic and uterine epithelial cell apposition and adhesion. The attachment is complete by day 35 to 40 post-fertilisation.

8. 6 Maternal recognition of the pregnancy

It has been well established that the ruminant embryo secretes substances some of which are protein in nature, which give the signal to the maternal uterus, thus preventing the production of the luteolytic factor, $PGF_{2\alpha}$. In the bovine, this factor is called bovine trophoblastic protein-1 (bTP1). How the bTP1 exerts its action is not exactly clear, but it is believed to be through specific receptors in the maternal endometrium and through inhibition of production of $PGF_{2\alpha}$.

8.7 Structure and function of the bovine placenta

The type of placentation as in other domestic mammals is chorio-allantoic and this is formed by fusion of the outer layer of the allantois to the chorion. The allantois is derived from the diverticulum of the hind gut and the chorion is derived from the trophoblastic capsule of the blastocyst. Structurally, the attachment is limited to special areas called *uterine currencles* and the foetal chorion at each point of attachment shows specialisation and these specialised areas are called *foetal cotyledons*. Foetal cotyledons and maternal currencles together constitute the functional unit of the placenta and this is referred to as the *placentome*. In both cattle and buffaloes, the gross shape of the maternal currencle is convex and it is estimated that the pregnant uterus possesses 70 to 120 placentomes. The bovine placenta is cotyledonary in type and epithelio-chorionic morphologically.

The placenta performs a multitude of functions and substitutes for the foetal gastro-intestinal tract, lungs, kidney, liver and also acts as an endocrine organ. In addition, the placenta separates the foetal and maternal organisation thus ensuring the development of the foetus in a protected and separate environment. The endocrine function of the placenta shows a great deal of species variation. In general, it is a transient endocrine organ like the corpus luteum of the ovary. It produces and secretes both protein (oxytocin) and steroid (progesterone) hormones. This endocrine activity is a cooperative function of foeto-placental unit, where both the foetus and mother act supplementing each other in hormone synthesis.

8. 8 Pregnancy maintenance and gestation

In cattle, the corpus luteum of pregnancy is essential for maintaining pregnancy until late gestation. According to more recent reports, the bovine placenta does indeed produce progesterone in the last half of gestation, contrary to the belief held earlier. Hence, ovariectomy after 200 days of gestation sometimes does not result in abortion. This in fact has practical implications in choosing a line of treatment for induction of parturition. The gestation length varies from 270 to 295 days in cattle, and buffaloes, it could range from 300 to 330 days. Both indigenous cattle and buffaloes tend to have longer gestation periods than their respective exotic counterparts.

8.9 Parturition

The scanty experimental evidence and experiences with clinical situations suggest that cortisol, presumably of foetal origin is the trigger for initiation of parturition, as has been proposed for sheep (Fig. 8.2). For example, exogenous corticosteriods, such as hydrocortisone and synthetic compounds such as dexamethasone or flumethasone could induce parturition, but more potent and long acting compounds yield better results. Prostaglandin is not very effective in inducing parturition, suggesting the existence of placental source of progesterone.

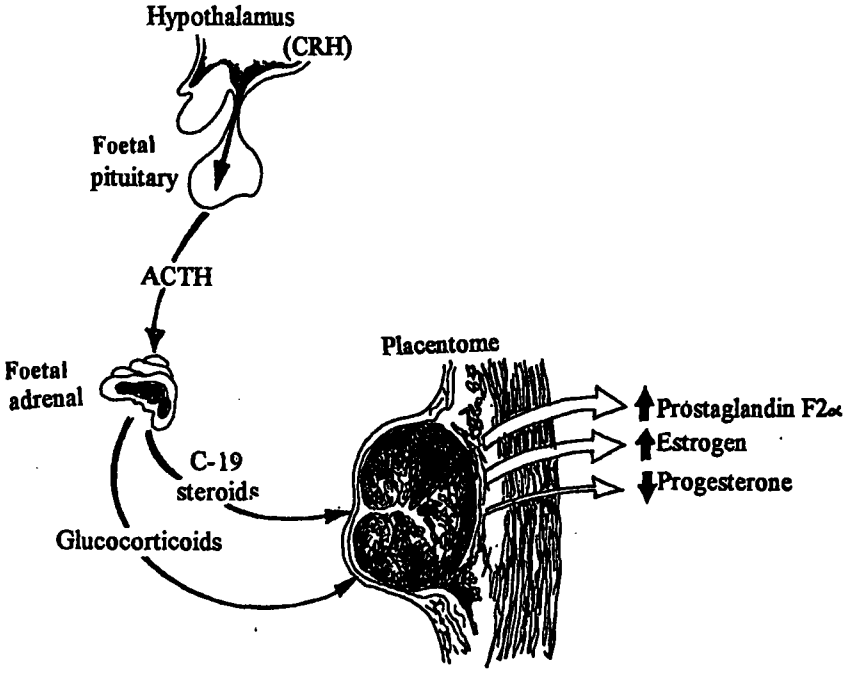

Fig. 8.2 Proposed model to explain the mechanism of parturition in sheep.

In the proposed model for cattle, it is assumed that foetal cortisol may induce rate limiting enzymes (17∝ hydroxylase, $C_{(17)}$ - $C_{(20)}$ - lysase, and aromatase) in the placental steriodogenesis pathway, causing the conversion of progesterone into oestrogen. The change in the progesterone to oestrogen ratio, then causes the release of phospholipase A2 from the lysosomes, which in turn causes the release of the substrate for $PGF_{2\alpha}$ synthesis, arachidonic acid from the cellular phospholipids. $PGF_{2\alpha}$ then acts on several sites. It acts on (a) the CL of pregnancy causing the regression and decline of progesterone secretion, (b) the proteoglycan matrix of collagen fibres of the cervix thus causing ripening and loosening of collagen bundles and (c) the smooth muscle cells of the uterus causing excitation and contraction.

The drop in progesterone (removal of the progesterone block) increases the excitability of smooth muscle cells (also due to oestrogen and $PGF_{2\alpha}$ effects) and the spontaneous contraction of smooth muscles ($PGF_{2\alpha}$ may stimulate contraction)

gradually pushes the foetus towards cervix, which is undergoing ripening and loosening. The reflex stimulation of the cervix then calls upon the Ferguson's reflex to release more and more oxytocin from the maternal posterior pituitary. Oxytocin then reinforces the maternal uterine contractions and precipitates maternal straining which further aids the passage of the foetus through the well lubricated maternal birth canal.

8.9.1 Signs of approaching parturition

In cattle, several clinical signs may indicate impending parturition.

- Softening and relaxation of muscles and ligaments of the rump, perineum and tail (oestrogen effects)
- Swelling of the vulva (oestrogenic effect)
- Discharge of thick, stringy mucus from vulva as a result of liquefaction of mucous plug of pregnancy
- Enlargement of udder and teats
- Change in behaviour - the cow may become restless as a sign of discomfort

8.9.2 Stages of parturition

Parturition or labour commences with the onset of regular progressive dilatation of the cervix. For descriptive purposes, the process of labour is divided into three stages.

8.9.2.1 *First stage of labour* - (dilatation of cervix) - duration 2-6 hours: The changes that occur during this phase is not externally visible, but they are very important because they prepare the birth canal for the smooth expulsion of the foetus. The changes that occur during this phase are:

- Change in physiological properties of the cervix ('ripening process')
- Onset of rhythmic myometrial contractions (also stimulated by $PGF_{2\alpha}$)
- Foetus assumes the correct disposition for expulsion - rotation about its longitudinal axis and extension of extremities

These externally invisible changes are accompanied by profound changes in the behaviour and physical status. The cow may become restless and show signs of abdominal discomfort. Other changes may include increase in pulse and respiratory rates.

8.9.2.2 *Second stage of labour* - (expulsion of foetus) - duration 0.5 to 1 hour: The commencement of this phase is marked by the onset of strong rhythmic contractions superimposed by abdominal contractions. These contractions may occur as frequent as 24 to 48 per hour, so that one contraction is almost immediately followed by another. These contractions are self-perpetuating with more and more release of oxytocin from the posterior pituitary. This is soon followed by the rupture of the chorio-allantoic membrane ("first water bag") with escape of urine-like fluid from the uterus. This is soon followed by the appearance of the amniotic membrane ("second

water bag"), which subsequently ruptures with increase in uterine pressure and abdominal straining. The rupture of the amnion marks the commencement of the delivery process of the foetus. Thick, slimy amniotic fluid provides lubrication for the easy passage of the foetus.

8.9.2.3 *Third stage of labour* - (expulsion of placenta) - duration 6-12 hours: The rhythmic contractions of the uterus which commence at the apex of the uterus continue until the placental separation (inversion and detachment) is completed and the placenta is expelled. This period is marked by a gradual cessation of maternal straining with simultaneous loosening of chorionic villi from maternal currencular crypts.

8.10 Post-partum period

The period following calving to the commencement of the first oestrous cycle is called the post-partum period. Lactation is initiated along with calving and the gravid uterus undergoes an extensive repair process (uterine involution) and the relatively inactive hypothalamo-pituitary-gonadal axis is reset to commence regular cyclic activity (resumption of ovarian activity).

8.10.1 Uterine involution

The previously gravid uterus must return to the non-gravid state in order to receive gametes at service and a conceptus following fertilisation. The process of getting rid of debris and contaminant bacteria, restoration of the uterine endothelium and reduction in size to the pre-gravid state is called uterine involution. As indicated earlier, this process involves several activities: (i) shrinkage of the myometrium and atrophy of cells; (ii) elimination of placental debris and contaminant bacteria and (iii) regeneration of endometrium.

During the immediate post-partum period, the secretion of uterine prostaglandin is high causing strong, rhythmic myometrial contractions, which aid the reduction in the size of the uterus. The rate of reduction is slow during the first 4 to 10 days post-partum and rapid thereafter, usually completing the process by day 25 to 30 post-partum. The uterine currencles undergo necrosis and this process is completed by day 5 or so and the currencular surface is denuded of necrotic tissue by day 10-12 post-partum. The regeneration of the epithelium starts from the edge of the currencle and it is usually completed by day 25 to 30 post-partum in a non-contaminated uterus. The placental debris and necrotic currencular material are expelled from the uterus as *uterine lochia* and the volume is greatest by 48 hours post-partum. This continues, but the quantity diminishes as the process advances, and by day 14, it may be about a few millilitres in quantity.

The bacteria, both pathological and non-pathological, that enter the uterus through the dilated cervix during the parturition process undergo multiplication under the favourable uterine environment. The natural defence mechanism of the involuting uterus is the infiltration of leucocytes to counteract this bacterial invasion. The increased myometrial activity with the onset of folliculogenesis, through oestrogens, further assists the uterus in eliminating the injurious agents through the cervix.

The post-partum uterus could become infected with pathogenic bacteria and the common organisms are *Corynebacterium pyogenes, Escherichia coli, Pseudomonas aeurogenosa, streptococci and staphylococci* species. They are usually eliminated by the uterine defence mechanism, which is further facilitated by the onset of post-partum ovarian activity. If the infection persists, the resulting uterine lochia, which is usually white-yellow in colour becomes mucopurulent in character. Several factors could affect uterine involution; uterine inertia, retained placenta, infections, old age and delayed ovarian rebound. In uncomplicated cases, the uterine involution process may be completed generally day 25 in cattle and by day 35 in buffaloes.

8.10.2 Factors affecting uterine involution

8.10.2.1 *Uterine infection*: Any inflammatory condition of the uterus such as placentitis (e.g. brucellosis) or infection following unhygienic calving or dystocia or caesarean operation or retained placenta could delay the involution process. The bacterial invasion and the resulting uterine infection could compromise the first line of uterine defence (i.e. killing of micro-organisms by leucocytes) and as a consequence an uterine pathology sets in. This uterine infection in turn delays the involution and also the resumption of ovarian activity, presumably acting through increased levels of $PGF_{2\alpha}$ released from inflamed tissues and also due to the presence of toxins. It has been shown that very seldom the resumption of ovarian activity occurs post-partum until $PGF_{2\alpha}$ levels reach basal concentrations.

8.10.2.2 *Old age*: Old cows usually have a senile uterus which is more prone to uterine infections. The musculature is weak and the uterine contractions which help to get rid of debris and contaminant microbes are impaired, and the uterus becomes more prone to infection. As a consequence, the involution process gets affected and causes delays both in involution of the uterus and resumption of ovarian activity.

8.10.3 Resumption of ovarian activity

In cattle, the CL of the previous pregnancy regresses completely by day 7 post-partum and the immediate post-partum period is characterised by the absence of steroids (oestrogen and progesterone) and low or undetectable levels of pituitary gonadotrophins in plasma (LH and FSH). As the post-partum period progresses, follicular development begins to appear and this is associated with the appearance of pulsatile LH secretion. The low levels of gonadotrophins in the circulation immediately after parturition is believed to be due to inhibitory affects of progesterone and oestrogen during pregnancy on the hypothalamo-pituitary axis. The recovery process from this inhibition is gradual and the appearance of pulsatile LH secretion signifies this rebound following parturition. It has been shown in many studies, that the resumption of this pulsatile LH (as well as FSH) secretion is a prerequisite for the onset of ovarian activity following parturition. This recovery process is usually completed by day 10 to 14 post-partum in lactating but non-suckled cows and ovulation could occur well before the completion of involution of the uterus. In the past, it was thought that involution of the uterus and resumption of ovarian activity are independent processes. However, in the light of more recent findings, it appears that these two processes are inter-related. According to these reports, extensive uterine

inflammation could delay postpartum oestrus and this is perhaps due continuous secretion of prostaglandins from inflamed tissues which could bring adverse affects on ovarian function directly, as well as indirectly through the higher centres such as the pituitary.

As in the case of uterine involution, the process of initiation of ovarian activity is also influenced by many factors. Therefore, the length of the post-partum period varies greatly both in cattle and buffaloes. The resumption of ovarian activity signified by the first oestrus could commence on an average, after 30 days in cattle and 45 days in buffaloes. But the process of resumption of ovarian activity could be influenced by (a) the age of the cow, (b) the calving season, (c) pre- and post-partum nutrition, (d) pre-partum and peri-partal diseases and (e) lactation and suckling.

8.10.4 Factors affecting resumption of ovarian activity

8.10.4.1 *Age of cow*: A relationship between the age at calving and subsequent resumption of ovarian activity seems to exist. It takes longer in heifers than in mature cows. This is because heifers may be subjected to inadequate nutrition following calving, particularly if they have been underfed during pre-and post-partum periods. The demand for nutrients is high in these animals, as they not only require nutrients for lactation but also for growth. On the other hand, old cows may also take a longer time to resume oestrous activity post-partum or become infertile as a result of many factors. There is a partly related reduction in the number of primordial follicles in the ovary with advancing age. Further, the senile uterus is more prone to infection and hence old cows may go through delays in uterine involution, and as a consequence, take a longer time to return to oestrus than young cows.

8.10.4.2 *Season of calving*: Fertility of cattle as well as buffaloes undergoes considerable variation with season. In high latitude areas (temperate regions), cows tend to have longer post-partum periods during short photoperiods than during longer photoperiods. In regions close to the equator (i.e. in the tropics), cows tend to have longer post-partum periods when calving occurs during the dry seasons when feed availability is low and ambient temperature and humidity are high. How these environmental factors cause alterations in the resumption of ovarian activity have not been fully understood as yet. However, it is conceivable that these adverse environmental factors may delay the rebounding of hypothalamo-pituitary gonadal axis from the pregnancy induced inhibition. These factors may have direct affects on the hypothalamic neural apparatus and also exert inhibitory affects indirectly through the secretion of stress related hormones such as cortisol, prolactin and endorphins which may act both at pituitary and gonadal levels. It has also been argued that both the cow and buffalo are seasonal breeders by nature, and when the environmental factors become limiting, they tend to manifest this inherent seasonality.

8.10.4.3 *Pre- and post-partum nutrition*: Many studies have shown that underfeeding during the pre-partum period as well as during the post-partum period results in delays in resumption of ovarian activity. It has also been shown that all major nutrients - energy, proteins, vitamins and minerals - are equally important in re-initiation of ovarian activity. However, how these different nutrients affect the

resumption of ovarian activity is far from complete understanding. Many studies lend support to implicate higher centre mediated actions in nutritional anoestrus, presumably inhibiting the resumption of pulsatile LH secretion following calving. A relationship between nutrition and body reserves, based on the body condition of animals, has been developed as a practical guide in feeding post-partum cows for optimum fertility (Chapter 28 on Body Condition Scoring System).

8.10.4.4 *Peripartal diseases*: Cows which have had abnormalities such as metabolic diseases (e.g. ketosis or hypocalcaemia) or post-partum uterine infections or generalised diseases tend to take a longer time to resume oestrous activity following calving. Some of these, such as post-partum uterine infections may have a direct affect on resumption of ovarian activity as discussed earlier, while metabolic disorders and generalised diseases may exert affects through disease on the nutrient status of animals, resulting deficiencies of major nutrients.

8.10.4.5 *Lactation and suckling*: Lactation demands more nutrients and as a consequence, if limitations in pre-and post-partum nutrition occurs, lactation could act as an inhibitor to the resumption of ovarian activity. In general, high yielding dairy cows tend to require a longer post-partum period than low producing cows. Studies have shown that lactation and suckling both inhibit the resumption of ovarian activity following calving. Greater inhibition occurs as the frequency of milking or suckling by the calf is increased. Experimental evidence implicates both tactile stimuli of milking and suckling as well as the cow-calf interaction as factors bringing about this inhibition and indicates that this is manifested endocrinologically through the delay in resumption of the pulsate LH secretory pattern following calving. How these inhibitory affects are brought about has been subjected to a great deal of scientific study, but the understanding is far from complete. It has also been shown that milking as well as suckling causes secretion of prolactin and cortisol while causing the alteration in neurohormones at the hypothalamic levels (e.g. CRH, endorphins, serotonin, etc.). It is conceivable that these factors may have direct as well as indirect affects on the function of hypothalamo-pituitary-gonadal axis and work in concert in an additive manner in bringing about the suckling induced post-partum anoestrus.

Selected references

Abeygunawardena, H., Ratnayake, D. and Jayatilake, M. W. A. P. (1997) Characteristics of cattle farming systems in Sri Lanka. *J. Natn. Sci. Coun., Sri Lanka.* **25,** 25-38.

Abeygunawardena, H., Abayawansa, W. D. and Perera, B. M. A. O. (1996) A comparative study of reproduction and productive characteristics of indigenous swamp and exotic river buffaloes in Sri Lanka. In: *The Role of Buffalo in Rural Development in Asia.* SAREC/NARESA Buffalo Research and Development Programme. pp: 297-308.

Abeygunawardena, H., Abayawansa, W. D., Ratnayake, D. and Jayatilake, M. W. A. P. (1994) *Zebu cattle farming in Sri Lanka: Production systems and reproductive characteristics.* IAEA-TECDOC - 736: pp: 37-51.

De Silva, L. N. A., Perera, B. M. A. O., Tilakeratne, L. and Edquvist, L-E. (1985) *Production systems and reproductive performances of indigenous buffaloes in Sri Lanka.* Monograph, Swedish University of Agricultural Sciences, Uppsala, Sweden.

McDonald L. E. and Pineda, M. H. (1989). *Veterinary Endocrinology and Reproduction* (4th edition). Lea and Febiger. Philadelphia, London.

Peters, A. R. and Ball, P. J. H. (1995) *Reproduction in cattle* (2nd edition). Blackwell Press, Oxford, UK.

CHAPTER 9

Male Reproductive System

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Introduction

The functions of the male reproductive system are to (a) produce the male gametes, spermatozoa, (b) produce sex hormones and (c) to transfer gametes into female reproductive system. The reproductive system is composed of the necessary organs and specialized structures to perform these tasks. The functions of these organs and structures are precisely controlled by the endocrine system as discussed in Chapter 7.

9.1 Structure of the male reproductive system

The male reproductive system of cattle and buffaloes, as in all the domestic animals consists of a pair of testes, epididymis, ductus deference and seminal vesicles on each side, the urethra, with the associated prostrate and Cowper's glands and the penis. The testes, their excretory duct system, including the pelvic urethra with associated accessory glands are referred to as the internal genital organs while the penis, prepuce and scrotum are the external genital organs. The urethra serves as a urogenital canal that conveys both urine and semen.

The general architecture of the male reproductive system of bovine is given in Fig. 9.1.

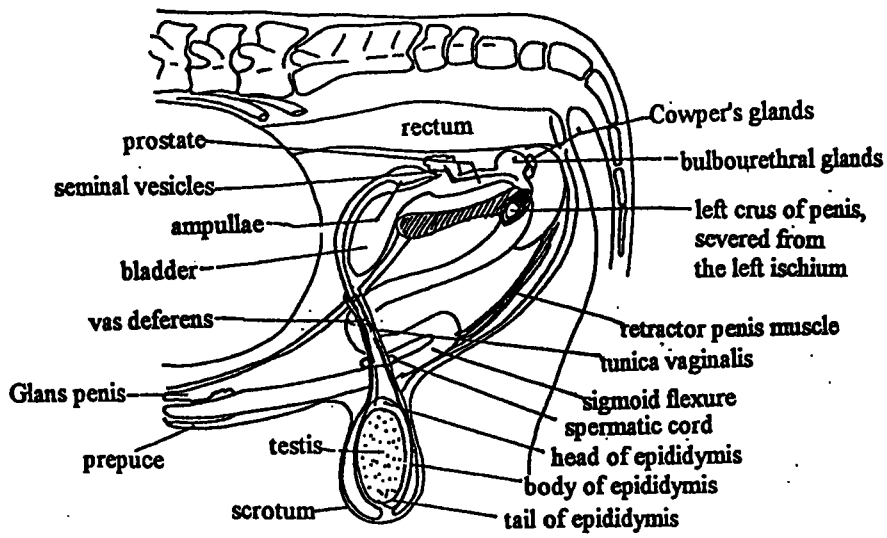

Fig. 9.1 Male reproductive organs of cattle. (Adapted from Peters and Ball, (1995)

9.2 Testes

Testes are paired organs, which descend during foetal life from abdominal cavity to lie in a pouch, the scrotum. The bilobed pendulus scrotum is located in the inguinal region. A substantial portion of the exocurrent duct system, e.g. epididymis and the beginning of the deferent duct, is also located in the scrotum. The two testes are similar in size and at adulthood each weigh about 350 gm in Bos taurine breeds. Testes are smaller outside the breeding season and in young animals. Spermatogenesis occurs throughout the year in bulls but seasonal depression of the production rate and fertility may occur.

Each testis initially develops as an abdominal organ, and descends subsequently towards inguinal canal to be located in a pouch, scrotum. As a result of this arrangement, the outer surface of testis is covered with peritoneum that is united with the underlying fibrous capsule, the tunica albugenia. From the deep layers of the tunica albuginea, numerous fibrous septula penetrates the testicular parenchyma and converge into a central core of connective tissue, mediastinum testis. The mediastinum testis is connected to the tunica albuginea of the proximal pole of the testis where the head region of the epididymis is attached. Hence, the testis or testicular parenchyma is divided into hundreds of conical lobuli and each made up of a limited number of highly convoluted, individual seminiferous tubules connected at both straight ends with a network of tubules, located at the mediastinum which is called the rete testis. From the rete testis some 10-20 efferent ducts arise, penetrate the tunica albuginea and converge into the head of the epididymis where they join to form the single epididymal duct (Fig. 9.2).

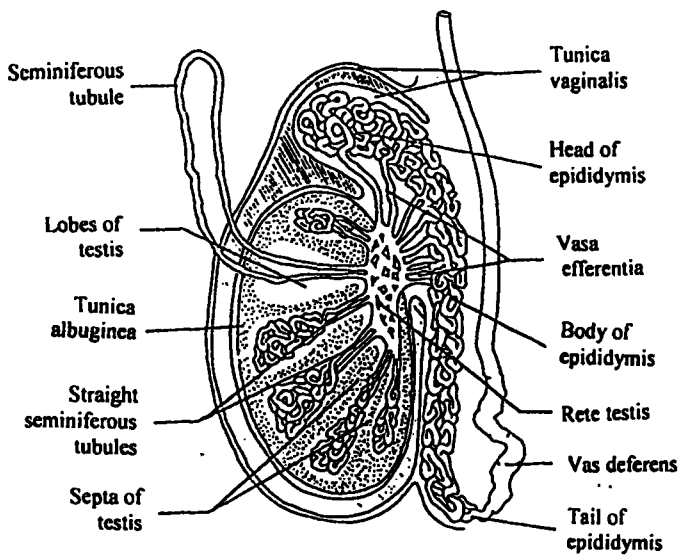

Fig. 9.2 Structure of testis. (Adapted from Setchel, 1978 and Johnson and Everitt, 1988)

The testicular parenchyma is largely made up of seminiferous tubules (about 80-90% of the volume). The rest of the testicular parenchyma is called interstitial tissue. These two tissues are organized into two compartments (Fig. 9.3); seminiferous tubular and interstitial tissue compartments. The seminiferous tubular compartment, which consists of germ cells and Sertoli cells, is specialized for production of gametes (Fig. 9.3a) while the interstitial compartment, which primarily consists of Leydig cells, produces androgens (Fig. 9.3b). The two compartments are not only distinct structurally but are also separated physiologically in the adult by a cellular barrier (by blood-testis barrier) that affects the free exchange of water soluble materials.

9.2.1 Seminiferous tubules

Seminiferous tubules are approximately 200-250 μm in diameter and it has been calculated that there are between 12-15 million tubules per gram of testis. Each tubule is essentially a long cylinder with numerous bends, forming a loop that opens at both ends into the rete testis.

The spermatozoa are formed within the seminiferous tubules, a protected environment, which is not penetrated by blood or lymph or by nerves. The cells inside the seminiferous tubules are the somatic Sertoli cells and the various germ cells and they are surrounded by a specialized peritubular tissue, consisting of layers of non-cellular material and myoid cells. These cells are contractile and cause peristaltic movements of the tubules which presumably are important for propelling the luminal fluid and the spermatozoa it contains out of the tubules. The lumen of each tubule develops only at puberty and contains a fluid that is quite unlike blood plasma or lymph in composition.

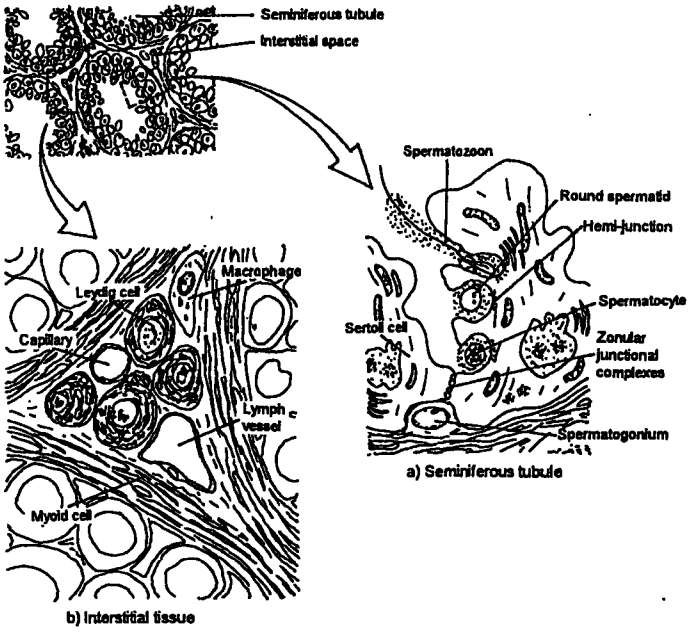

Fig. 9.3 Cross section of a testis depicting seminiferous tubules and interstitial tissue. (Adapted from Setchel, 1978)

The space between the seminiferous tubules is referred to as the interstitial tissue and it is here that the blood vessels, lymph vessels and nerves are found, with the androgen-producing Leydig cells and other cell types such as macrophages, and in some species mast cell (Fig. 9.3b). Most of the Leydig cells are situated either in close association with the blood vessels or close to the tubular walls. Leydig cells make up a much greater fraction of the interstitial tissue so that blood and lymph vessels appear comparatively indistinct.

9.2.4 Testicular capsule

The parenchyma of the testis is contained within a tough fibrous capsule, the tunica albuginea. This consists of contractile elements and connective tissue. Contraction of the capsule may be important in maintaining fluid transfer out of and back into the blood vessels.

9.3 Epididymis

The epididymis basically consists of a single tube tightly coiled and held together by a tough capsule. It lies over the length of the testis firmly attached to its medial surface. Conventionally, the epididymis is divided into the caput, corpus and cauda epididymis. The head region (caput) is apposed to the proximal pole of the testis, where the epididymal duct arises from the efferent ducts, the slender body (corpus) terminates in the expanded tail region (cauda) at the distal pole of the testis. The contour of the cauda epididymis is a visible and palpable feature in the live animal. The tail finally tapers and the duct emerges to continue as the different duct. Around the epididymal duct there is a smooth muscle that is progressively developed from caput and continues over the deferent duct. Peristaltic contractions of this muscle layer occur during ejaculation. In addition to providing the means of transport of spermatozoa from the testis to the deferent duct, the epididymis also functions to concentrate, mature and store the male gametes.

9.4 Deferent duct

After leaving the tail of the epididymis, the deferent duct courses over the medial side of the epididymis towards the proximal pole of the testis, where it becomes incorporated in the spermatic cord together with blood vessels and lymph vessels of the testis. The spermatic cord is attached to the inside of the narrow neck of the vaginal process and forms a conical structure between the vaginal ring and the proximal pole of the testis. Upon entering the abdominal cavity, the deferent duct curves toward the pelvic cavity where it enters the dorsal wall of the pelvic urethra. The wall of the deferent duct contains an ample amount of smooth muscle arranged in an inner circular and outer longitudinal layers.

9.5 Accessory genital glands

The full set of accessory genital glands comprises paired ampullary, vesicular, prostrate and bulbo-urethral glands. All accessory glands are present in the cattle and buffaloes. The glands have well developed capsules and internal septa in which there

is much smooth muscle. Upon contraction of the muscle cells during ejaculation, the mucous secretion of the branched tubulo-alveolar glands is expelled into the urethra. The secretions cleanse the urethra, lubricate the penis and vagina and neutralize the acidic vaginal environment, and also provide fructose as an energy source for ejaculated spermatozoa.

The position of the accessory reproductive glands within the pelvic cavity makes them accessible for examination by rectal palpation. All male accessory glands are sensitive to steroid hormones and increase in size when stimulated by steroids.

Ampulla: It is an enlargement of the terminal portion of the deferent duct. It contains typical branched, tubular glands with sac-like dilatations.

Seminal vesicles: Seminal vesicles develops from the distal part of the deferent duct in the embryo and this relationship persists in adult ruminants. The glands lie wholly or partly within the genital fold, each lateral to the corresponding deferent duct. The vesicular glands in cattle are compact lobulated glands with rather narrow branched lumina. The thick wall contributes to the knobbly appearance of the glands in ruminants.

Prostate gland: It consists of two components; one diffusely spread along the length and within the wall of the pelvic urethra and the other a compact body placed on the dorsal aspect of the urethra, outside the urethral muscle. Both parts are drained by many small ducts.

Bulbo-urethral gland: It is a gland which is paired and lies in the dorsal aspect of the pelvic urethra close to the pelvic exit. The glands are small and globular in structure.

9.6 Scrotum

The scrotum is the sac that contains the testes. The scrotum is derived from the abdominal wall and is composed of several layers of tissue - skin, the tunica dartos muscle and the external spermatic fascia. The relatively thin scrotal skin is well provided with sebaceous and sweat glands and lacks subcutaneous fat. The scrotum serves as a protective covering for the testis. Moreover, it has an important role in thermoregulation of the testis. Temperature within the testicular parenchyma is maintained at approximately 5°C below body temperature for effective spermatogenesis. Heat loss by radiation is facilitated by the exposed position of the scrotum, the absence of fat within the scrotal fascia and the intra-capsular position of large testicular vessels. Additional heat loss by evaporation is allowed by the generous supply of sweat glands. Counter measures to prevent excessive heat loss are also available. Contraction of the tunica dartos directly sensitive to temperature change, tightens and bunches the scrotum, thereby reducing the exposed surface and also elevating the testes towards the warmer trunk. In hot conditions, the tunica dartos relaxes, lowering the testes within the thin-walled pendulous scrotum.

9.7 Vaginal tunic and spermatic cord

Vaginal tunic: This is the peritoneal process that encloses the testes, and its adnexa (vaginal process) is an evagination of the lining of the abdomen through the inguinal canal. The vaginal cavity between the perietal and visceral layers of the vaginal tunic normally contains a minute amount of serous fluid. It communicates with the peritoneal cavity of the abdomen through the vaginal ring.

Spermatic cord: The spermatic cord contains deferent ducts, arteries and veins, lymphatic vessels, pampiniform plexes, external cremaster muscle and vaginal tunic. Upon entering the abdominal cavity the spermatic cord parts into the mesorchium (peritoneal folds that suspend testes and its adnexa) and the pica ductus deference which follow different courses. Left and right plicae are continued into the pelvic cavity where they fuse just dorsal to the neck of the bladder to form the genital fold. Each of the left and right parts of the genital folds contain a vesicle gland, ureter and the deferent duct.

9.8 Penis and prepuce

The penis is the male copulatory organ and is made up of the urethra and three columns of erectile tissue which during erection engorge to give sufficient stiffness to the penis to be introduced into the female copulatory organ.

The prepuce or sheath is the inverted fold of the skin that surrounds the free tip of penis. The inner hairless part is well provided with smegma-producing glands.

9.9 Sperm transport and ejaculation

Spermatozoa are immotile when released into the lumen of the seminiferous tubules. They reach the epididymal duct in a passive way, floating in the current of fluid secreted by the Sertoli cells. Progress through the epididymis most probably depends on spontaneous peristalsis of its muscular coat. As in most species, the capacity for coordinated movements of spermatozoa is acquired by the time they reach the tail of the epididymis. Peristalsis of the deferent duct moves the spermatozoa towards the ampullary region. In sexually inactive animals, spermatozoa are lost from here through seepage into the urethra. The seepage is regular but emission is slow in contrasts to the vigorous emission during ejaculation.

9.10 Blood, lymphatic and nerve supply

Blood supply: The testes and epididymis receive the blood supply from the testicular artery originating from the aorta in the midlumbar region. The intra-pelvic organs (deferent duct, prostrate, ampulla, vesicular gland and bulbourethral gland) receive the blood supply from the branches of the internal iliac artery. The penis receives blood from the branches of internal pudental artery. The external genital organs (scrotum, prepuce) receive blood from the branches of external iliac artery.

A special arrangement exists between the testicular artery and the testicular vein. In its distal course within the spermatic cord, the testicular artery becomes highly convoluted, coils being surrounded by a fine mazed meshwork of the testicular veins. The venous meshwork is the so-called pampiniform plexes. This arrangement provides a large contact surface between artery and the vein and also a counter current mechanism. It is believed that heat exchange takes place between the warmer arterial blood and the cooler venous blood, aiding in keeping the testes at lower than body temperature.

Lymphatic supply: The prepuce, scrotum and penis, including the associated muscles and the penile urethra are drained by the superficial inguinal lymph nodes. These nodes are located in the suspensory facial structures dorsal to the penis. In ruminants, they are located near the neck of the scrotum and hence are sometimes referred to as scrotal lymph nodes. The superficial inguinal lymph nodes drain towards the deep inguinal lymph nodes, which also receive lymph directly from the penis and from the vaginal process and the associated cremester muscle. The pelvic urethra and associated structures like the root of the penis and the accessory glands drain towards sacral lymph nodes. Ultimately the medial iliac lymph node, situated around terminal bifurcation of the abdominal aorta just dorso-cranial to the pelvic inlet receive lymph from the testis, epididymis and deferent duct and from the accessory glands. Medial iliac lymph nodes and sacral lymph nodes belong to iliosacral lymph centre, which ultimately receive all lymph from the genital tract and drains into cisterna chyli *via* the truncus lumbalis. Inflammation of the free end of the penis and the prepuce occur frequently and may be associated with swelling of the superficial inguinal lymph nodes and further stations of lymphatic drainage. Inflammation of testes, its adnexa, and/or the accessory glands result in swelling of the medial iliac lymph nodes which can be examined by rectal palpation.

Nerve supply: The internal genital organs are supplied with autonomic nerves that travel alongside the blood vessels that supply the organs. The erectile components predominantly have parasympathetic innervation. External genital organs have somatosensory and somatomotor innervation supplied by the spinal nerves.

9.11 Spermatogenesis

Spermatogenesis is the process of formation and liberation of spermatozoa from undifferentiated germ cells in the testis. The process is functionally divided into (a) spermatocytogenesis and (b) spermeiogenesis.

9.11.1 Spermatocytogenesis

At the time of gonadogenesis the primitive germ cells migrate from the medoderm and colonize in the gonadal ridge on the edge of mesonephros. They multiply by mitotic division, become enclosed in cords of Sertoli cells, and then division ceases until puberty. Immediately preceding puberty (pre-pubertal period), the germ cells migrate between Sertoli cells and the wall of the tubule and from then on are known as spermatogonia (Fig.9.4). Some of them remain as non-dividing or slowly dividing stem cells (spermatogonia Ao or As). Others divide more frequently and become A1,

A2 and A3, Intermediate (In), B1 and B2 spermatogonia. The distinction between A, In, and B is based on the appearance of the nuclei and also on other cells with which they are invariably associated. The B spermatogonia then divide once more to become primary spermatocytes. These cells then undergo the first meiotic division and become secondary spermatocytes, which very soon afterwards pass through the second meiotic division to become spermatids. Spermatids are haploid cells that transform into spermatozoa over a period of about 15 days (spermeiogenesis) . One of the more remarkable features of spermatogenesis is that all divisions of the cells from A1 spermatogonia onwards are incomplete in so far as cytoplasmic separation does not occur, and the cells remain linked by cytoplasmic bridges. Thus A1 spermatogonia give rise to a chain of 32 B2 spermatogonia, 64 primary spermatocytes and 256 spermatids.

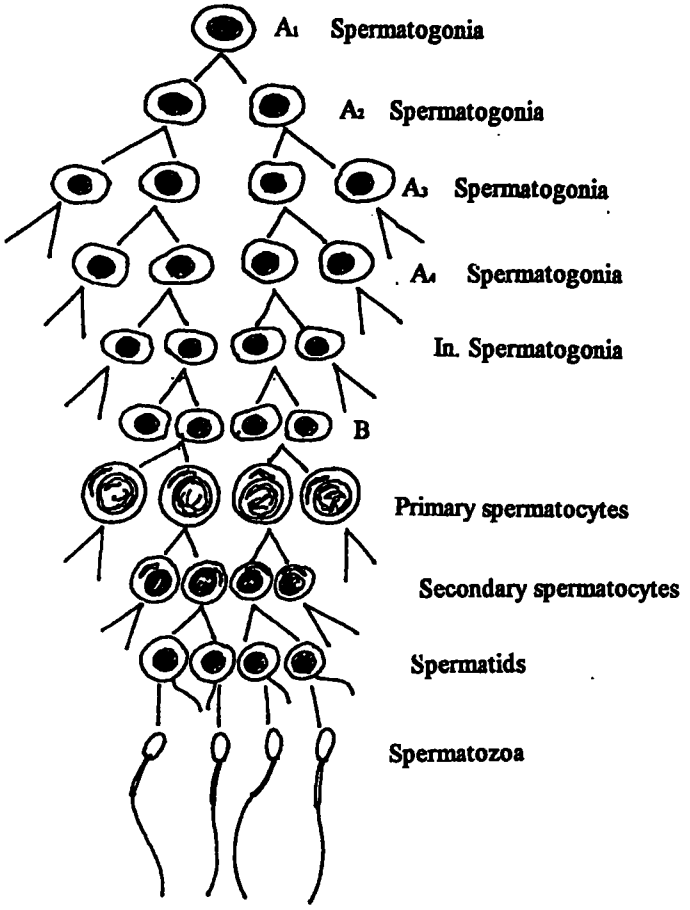

Fig. 9.4 Diagramatic presentation of spermatocytogenesis. (Adapted from Setchel, 1978)

9.11.2 Spermeiogenesis

This is the process of transformation of the newly-formed haploid spermatids into spermatozoa and this involves a remarkable transition from ordinary looking round cells with an undistinguished nucleus into highly organized spermatozoon (Fig. 9.5). Fifteen different steps in this process have been identified by various workers.

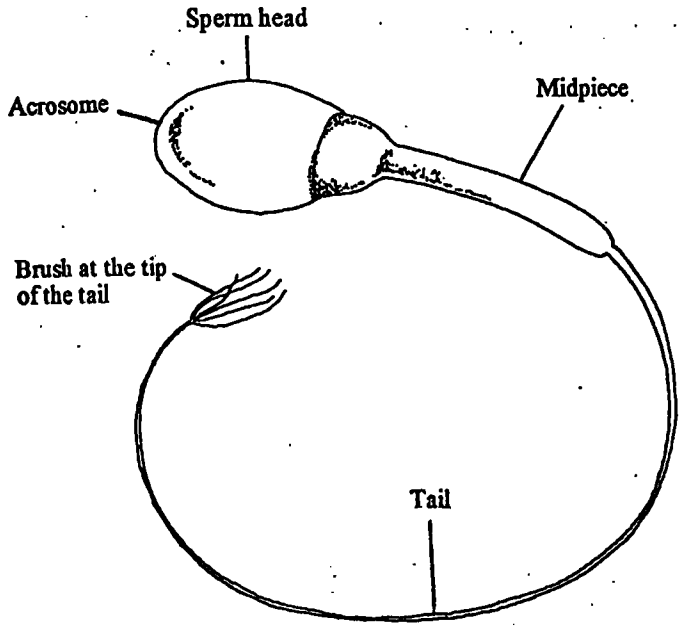

Fig. 9.5 Morphological structure of a sperm.

9.11.3 Function of Sertoli cells

Sertoli cells play an absolutely pivotal role in spermatogenesis. They do not appear to divide in the adult. The cytoplasm extends from the inner layer of non-cellular material of the peritubular tissue to the lumen of the tubule. They have a lobed nucleus of very characteristic appearance, hold the germ cells either in crypts in their luminal surface, between one Sertoli cell and its neighbour or between one Sertoli cell and the peritubular tissue. Once the germ cells enter the meiotic prophase, they are separated from the peritubular tissue by Sertoli cells, and during leptotene, a new series of specialized junctions form between pairs of Sertoli cells, where cytoplasm meets. These junctions are part of the blood testes barrier and probably ensure that the germ cells after leptotene develop in isolation from the rest of the body. The germ cells remain between pairs of Sertoli cells during their meiotic prophase, through the meiotic divisions and the early stages of spermatogenesis, while there is an older generation of spermatids still attached to the Sertoli cells. However, when these older spermatids are released, the young spermatids move around to the luminal surface of the Sertoli cells and undergo the rest of their development in crypts in the luminal surface.

9.12 Dynamics of spermatogenesis

One of the remarkable features of spermatogenesis is that the duration of the whole process and the timing of the various steps is apparently constant in any one species, and furthermore, the various steps are closely synchronized with other events in the process so that certain cells are only found in association with certain other cells, and never with others. This has given rise to the concept of the spermatogenic cycle (i.e.

the cyclic entry of spermatogonia into spermatogenesis). The cyclic entry of spermatogonia into the spermatogenesis process leads to spatial arrangement of cell associations across the cross section of the seminiferous tubule and this is referred to as the cycle of seminiferous epithelium. The length of the seminiferous epithelial cycle is characteristic for each species. For example, it is 14 days in the bull and 16 days in man. The duration of spermatogenesis can be calculated from the length of the cycle. For example in the bull, it takes approximately 4 cycles to complete the process; approximately 56 days from the appearance of A type spermatogonia to the release of a spermatozoon into the semineferous tubular lumen. In some species, there is also a spatial arrangement of phases of the cycle along each tubule, starting at the rete. This is referred to as the spermatogenic wave .

Theoretically, each A1 spermatogonia should give rise to 256 spermatozoa in the ram, because of the number of generations of germ cells involved in this species. In fact, this number is never attained because some germ cells always degenerate. It is not known whether this involves single cells or a whole clone, but it is much more marked at the end of the breeding season.

9.13 Disruption of spermatogenesis

Spermatogenesis can be disrupted in a variety of ways. Some of the factors are discussed below.

9.13.1 Environment

The scrotal testes of domestic animals are susceptible to damage if the temperature is raised to blood temperature or above. This could occur as a result of raised environmental temperature, possibly during febrile illness or because of the failure of one or both testes to descend normally from the abdominal cavity (e.g. cryptorchidism). This condition is not uncommon in the horse, pigs, and dog, but rare in cattle and sheep. It has been shown that in the ram even exposure of testes to moderately high temperatures (e.g. 42^{o} C) for shorter periods (e.g. twice 6 hourly) resulted in temporary infertility with changes in the numbers and morphology of sperm. The most susceptible to heat are cells in meiotic division. Studies have shown that bulls are more susceptible than rams in heat induced infertility. It has also been shown that heat-exposed spermatozoa, though they are capable of fertilizing ova, results in the death of embryos subsequently, thus increasing incidences of early embryonic death. Exposure of testes to cold appear to be less damaging than exposure to heat. This is because animals could maintain the temperature by pulling the testes up close to the body wall.

9.13.2 Radiation

Testis is also extremely sensitive to the effects of ionizing radiation such as X-rays and gamma radiation. In this case, spermatogonia are more affected than the cells of latter stages.

9.13.3 Nutrition

Studies have shown that underfeeding results in the reduction in testes size and this is more marked compared to the weight loss. This appears to be more related to effects of undernutrition on the endocrine system than directly on spermatogenesis. If the weight loss is sufficiently large, then fertility can also be impaired. Both energy and protein appear to be important. Among the micronutrients, Vitamin A and Zinc appear to produce major effects. Testicular tissues are very sensitive to Vit. A deficiency similar to retinal tissue.

9.13.4 Chemicals

Spermatogenesis has been shown to be affected by many chemicals. A few of the potent ones are dichlorobromopropane (soil nematocidal cpd.), sulphosalazine (antimicrobial against ulcerative colitis in human), gossypol (male contraceptive used in China) and cadmium.

9.13.5 Congenital abnormalities

Several abnormalities, developmental or cytogenic, have been shown to have an effect on spermatogenesis. Examples of such conditions are congenital hypoplasia of testes and testicular feminization.

9.14 Endocrinology of testis

Testes are very active endocrine organs. The principle hormone is testicular androgens. Besides that testes produce several other steroids, peptide hormones such as Mullerian inhibiting factor and inhibin (see Chapter 7 for more details).

9.15 Endocrine control of spermatogenesis

Studies have clearly demonstrated that the pituitary control testicular function through FSH and LH. In these studies, it has been shown that FSH specifically binds to Sertoli cells while the LH specifically binds to Leydig cells. However, the roles of the two gonadotropins in spermatogensesis and the precise mechanisms through which these hormones and others control spermatogenesis have not been well deliniated. There appears to be species differences with regard to the importance of each hormone in maintaining testicular function. The information is very scanty too, particularly with regard to farm animal species (see Chapter 7 for more details).

9.16 Maturation of spermatozoa

When spermatozoa leave the rete testes, they are immotile and incapable of fertilizing an ovum. Both of these functions develop during the stay in the epididymis. Spermatozoa from rete testes pass through the different ducts into the epididymis where they mature and are stored before ejaculation through the ductus deference. Spermatozoa take between 10-14 days to pass through the epididymis and sperms not ejaculated appear to trickle along the ductus deference and are then voided in the

urine.

The precise changes which occur in the sperm during the passage along the epididymis are largely unknown and available evidence suggest that the sequential changes in the epididymal luminal fluid probably play an essential part in sperm maturation. The changes in composition of sperms are remarkable in this regard. There are changes in the types and pattern of proteins in the plasma membrane. Some of these appear to be taken up from the epididymal fluid, while the other changes may appear to be the masking and unmasking of membrane proteins by the addition or removal of other proteins or by the action of enzymes in the luminal fluid on proteins on the sperm surface. There are also significant changes in the amounts and nature of phospholipids and the neutral lipids in the spermatozoa. As the sperms lack ability to synthesize most of these compounds, the changes must be imposed by the luminal environment. One noticeable change is the movement of cytoplasmic droplet (i.e. remnant of cytoplasm left behind on the sperm when it is shed from Sertoli cells). It moves along the mid - piece from just behind the head towards the junction of the mid-piece and the tail and finally is shed off completely.

Table 9.1 Some details of the composition of semen of cattle

Constituent/Parameter	Cattle
Semen	
Volume (ml)	2-10
Dry weight (%)	9.5
Sperm concentration (x 10^{-6} / ml)	300-2000
Spermatocrit (%)	10
pH	6.48-6.99
Specific gravity	1.035
Seminal plasma	
Protein (gm/100 ml)	3-8
Fructose (mg/100 ml)	120-540
Sorbitol (mg/100 ml)	10-136
Citric acid (mg/100 ml)	357-1000
Ascorbic acid (mg/100 ml)	8.7
Inositol (mg/100 ml)	25-46
Ergothioneine (mg/100 ml)	Trace
Glutamic acid (mg/100 ml)	35-41
Glycerylphosphorylicholine (mg/100 ml)	110-500
Sodium (Mmol / l)	117
Potassium (Mmol / l)	44
Calcium (Mmol / l)	9.3
Magnesium (Mmol / l)	3.4
Chloride (Mmol / l)	49
Bicarbonate (Mmol / l)	7.1(WS)*
Mannosidase (units / ml)	400
N – Acetylglucosaminidase (units / ml)	15000

Source: Cole and Cupps (1977).

9.17 Semen

Spermatozoa are expelled, bathed in secretions from the testes and accessory sex glands at ejaculation. Ejaculation is a nerve mediated series of contractions of smooth muscle surrounding the cauda epididymis, the ductus deference, various accessory glands and the urethra, which expel a mixture of spermatozoa and the secretions of the accessory gland into the female tract. This mixture is called semen.

A summary of the more important constituents of the semen of cattle is given in Table 9.1. The concentration of fructose in the seminal plasma has often been used as an indicator of the androgen status of the animal as its secretion is strictly governed by the level of androgens in the blood.

9.18 Semen morphology

Semen contains spermatozoa and various other cell types. The morphology of sperms and the number and types of other cell types found in a sample of semen is used as criteria in evaluating the quality of semen. Cell types usually seen in a microscopic field of a semen sample are shown in Fig. 9.6. For more details see Chapter 10.

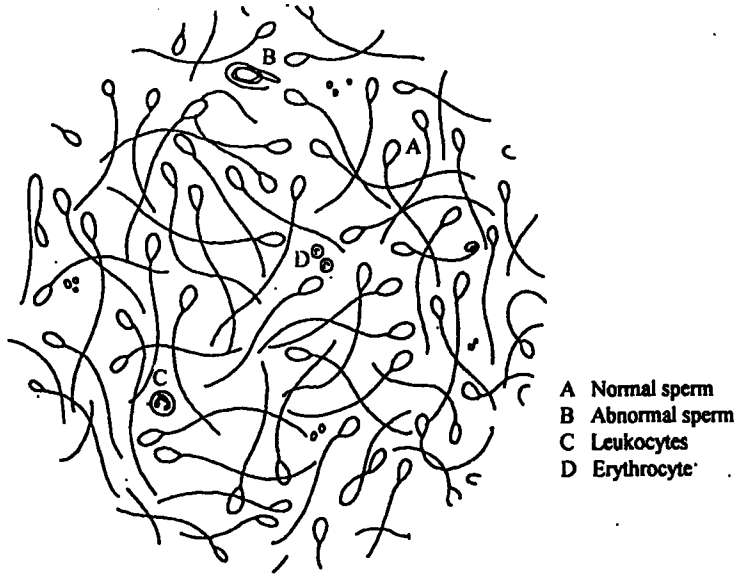

Fig. 9.6 Cell types in a semen sample. (Adapted from Peters and Ball, 1995)

Selected references

Cole, H.H and Cupps.P.T. (1977) *Reproduction in Domestic Animals.* (3rd edition). Academic Press, New York, USA.

Laing, J.A., Brindley Morgan, W.J. and Wagner, W.C. (1988) *Fertility and Infertility in Veterinary Practice.* (4th edition), Bailliere Tindall, London

Peters, A.R. and Ball, P.J. (1995) *Reproduction in Cattle.* (2nd edition). Blackwell Science Ltd. Oxford, UK.

Setchel, B.P. (1978) *The Mammalian Testis.* Cornell University Press, New York, USA.

CHAPTER 10

Bull and Semen Evaluation

H. Abeygunawardena

Introduction

Sexually active and fertile males, the competence of the technician and fertile semen are factors of paramount importance in achieving high conception rates in a cow population. Very often Veterinarians tend to ignore the male component of infertility and tend to direct the investigations and then the remedial measures towards the female only.

10.1 Bull evaluation

The bull or semen must always be suspect when confronted with a case of repeat breeding of pluriparous cows. In routine practice as well, bulls should be examined for breeding soundness before they are used (1) for natural breeding, (2) in artificial breeding programmes, (3) for insurance purposes or (4) when infertility is suspected. The examination is aimed at establishing whether or not (a) the animal is normal with respect to its general condition and health (b) it has normal reproductive organs, (c) they are functionally normal and (d) there are any abnormalities in behaviour. The first step in this regard is to establish a diagnosis and the second step is to determine the severity of the condition. The Veterinarian must keep all records of examinations as these will form a part of the report or certificate.

The examination consists of 4 aspects: (i) a general clinical examination, (ii) physical examination of the reproductive organs, (iii) examination of serving ability and serving capacity and (iv) semen evaluation.

10.1.1 General clinical examination

This procedure is the same as that adopted in the examination of a sick animal with a medical condition. The first step is to obtain the clinical history - details of breeding, origin, management, nutrition, environmental changes, general health from birth to the present state and the previous reproductive performance. Following application of adequate restraining methods, an extensive examination must be carried out, employing appropriate clinical examination methods of inspection, palpation, percussion and if necessary, laboratory examination of tissue samples. The gait of the animal should be observed, if possible from the front, the side and behind. The serving ability and serving capacity tests described later in this chapter also will give a good indication of the soundness of the back, the legs and feet.

10.1.2 Physical examination of the reproductive organs

The reproductive organs that must be examined are – the scrotum and its contents, penis, prepuce, seminal vesicles, ampullae and prostrate.

10.1.2.1 *Scrotum*: Palpation of scrotal contents is done by standing or squatting directly behind the bull (after carefully restraining the animal). The scrotum should be inspected to detect asymmetry and skin lesions. The cords are grasped with both hands high up near the inguinal canal and hands are then moved down over the organs to determine the similarity of the two halves (i.e. two testicles) with respect to size, form, consistency and mobility. The size of the testes is measured at the greatest scrotal circumference keeping the testicles side by side in the scrotum. If there is considerable difference between two testicles, the length, breadth and thickness of each should be estimated.

The consistency of the testicles and epididymis must be determined separately. This is done by lightly tensing the skin over the testicle with one hand held dorsally and using the fingers of the other hand to detect details of consistency. The head, body and tail of the epididymis are then palpated. There are two components of consistency; i.e. firmness and resilience. Firmness is measured by the distance one can push the testicular tissue by application of pressure with the fingers. Resilience is measured as the amount of pressure on the palpating fingers, as the testicular tissue springs back on relaxation of the pressure on the tissues (pushes the palpating finger outwards). Firmness is graded from 1 to 5 as follows: 1= very firm, 2 = firm, 3 = moderate, 4 = soft, 5 = very soft. Resilience or springiness is graded from 1 to 5 as follows: 1= very high, 2 = high, 3 = moderate, 4 = low, 5 = very low. The score for firmness is given first followed by the score of resilience, and by convention the score for the left testicle is recorded first.

10.1.2.2 *Epididymis*: The size and consistency of the epididymis depends on its physiological or pathological state. The size and consistency of epididymal tails in particular reflect sperm production and storage. A large and firm epididymis suggests an inflammatory or fibrose condition. A moderately sized, firm and resilient epididymal tail that feels "full" tends to reflect good testicular function. Softness or lack of resilience would suggest poor sperm production.

10.1.2.3 *Prostrate and seminal vesicles*: Having completed the external palpation of organs, the examiner must now proceed to palpate the organs per-rectally. First, the location of the cylindrical pelvic urethra is established. It may undergo rhythmical contractions on palpation. Near its anterior end, is a ridge which is the prostrate gland. All these structures are very firm in consistency.

From the prostrate, the two seminal vesicles diverge. They are of variable size according to age and individual variation. In young bulls, one or two years of age, these glands would be 6-8 cm long, 1.5-3 cm wide and 1-2 cm in thickness. In bulls, 2-4 years old, the estimated measurements of the normal seminal vesicles are 8 to 10 cm x 2.5 to 3.5 x 1.5 to 2.5 cm respectively, in length, width and thickness. In older bulls, these glands may measure up to 15 cm x 3 to 4 cm x 2 to 3 cm. The consistency also varies with age. In young bulls, it tends to be soft, approximately similar to the consistency of the fleshy tissue at the lateral edge of the palm of the hand. Slightly firm and firm are terms used to describe an increasing firmness that develops as bulls gets older. The fourth grading, very firm is only found in pathological conditions. Pathological areas may be detected by consistent identification of firm areas among

soft glandular tissue. In young bulls that are not used to rectal examination, the glands often feel firm when the hand is first introduced. After the bull relaxes, the consistency may seem softer. In normal glands, lobules are well demarcated and soft in consistency. In some pathological glands, the lobules are more clearly defined and enlarged, while in other cases they are less clearly defined. The seminal vesicles are also fairly mobile in the normal bull. They can be picked up and manipulated in the anterior and middle parts. Mobility is lost when adhesions form as a result of inflammation. Marked asymmetry of the seminal vesicles should also be viewed with suspicion.

10.1.2.4 *Ampullae*: The ampullae can be detected between the seminal vesicles as two pencil-like converging bodies. The fore-finger or the second finger moved across the area helps to detect their presence, thickens and consistency. They can be picked up between fingers and thumb, and allowing them to slip out again greatly helps to the estimate the diameter and symmetry of the ampullae.

10.1.2.5 *Penis and prepuce*: The penis and prepuce are best inspected at service or when the bull serves into an artificial vagina. In these situations, the functional as well as structural abnormalities can be detected. If service is not observed, the penis must be manually protruded and examined. Many bulls do not object to simple manual protrusion of the penis. It is easier to protrude the penis while the bull is defecating or while there is an assistant's hand in the rectum. If the penis cannot be protruded, the abnormality responsible can generally be felt by external palpation.

10.1.3 Examination of serving ability and serving capacity[1]

Serving ability is defined as the desire and ability to serve a cow, while the serving capacity is a measure of behavioural efficiency. This is assessed by the number of services a bull achieves under paddocked conditions.

10.1.3.1 *Serving ability:* The serving ability is examined as part of the examination for breeding soundness. For this test, a suitable teaser restrained in a serving bail or crate is necessary. A cow in natural oestrus or a nymphomaniac cow or a cow brought into oestrus by artificial means could be used as the teaser. However, it must be remembered that the quality of the teaser has a considerable bearing on the behaviour of bulls. A teaser that is restless, twitches the tail and moves away as the bull approaches to mount, will put off some bulls. Behaviour is also influenced by the presence of people, sudden movements by the assistant or veterinarian while collecting semen into an artificial vagina. Dogs, vehicles and distracting elements will affect behaviour. The serving behaviour can be stimulated by allowing a bull to watch another bull mounting the teaser, or by another bull or the teaser mounting the individual under examination. Such sexual preparation will result in a more reliable assessment of the serving ability. It is also important that care should be taken to prevent the spread of venereal disease when examining animals for serving ability.

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1 Galloway (1993)

The test for assessing the serving ability in an individual should last at least 10 minutes. Some heavy bulls might mount the teaser after as much as half an hour with rather little evidence of sexual stimulation. If no results are obtained, a record should be made of the interest shown by the bull and whether it did serve under the given test conditions. The record will have to be interpreted in the light of the factors mentioned above and other findings of the examination, including the clinical history and the performance of other bulls under the same conditions.

If a bull does not attempt to serve, then a detailed record of the serving behaviour can be made by observing and scoring the following facets. The test may be performed with a teaser cow in heat or cow not in heat or with a bull or steer. The bull may be led by the herdsman or served in a yard. These conditions must be noted when interpreting the results.

(a) Libido (graded from 0-6)

0 = no interest in cow despite encouragement to serve.
1 = slight interest to mount with sniffing; or feeble attempt to mount.
2 = mounts with hesitation or after persistent efforts by attendant.
3 = mounts relatively willingly, without manifesting a strong desire.
4 = goes briskly forward and serves; mounts without hesitation; interest only concentrated on the cow.
5 = very willing to mount; very keen; strives hard all the time to come to the cow.
6 = impetuous and unbalanced; rushes up to cow and mounts whether penis is erect or not; frenzied.

(b) Erection during seeking or stiffness of penis (graded from 0-4).

0 = no erection after mounting.
1 = very poor erection; penis slack sometimes with very transitory stiffening.
2 = weak erection; penis straight all the time but stiffness not adequate except for more complete filling but transitory in nature.
3 = good erection; penis constantly stiff.
4 = very good erection; penis hard and straight at all times during seeking.

(c) Protrusion during seeking (graded 0-5)

0 = no protrusion of penis from prepuce.
1 = short protrusion (1-2cm)
2 = relatively good protrusion (to 10 cm).
3 = normal protrusion (to about 5-20cm).
4 = abnormally long protrusion.
5 = deviated (give description of deviation).

(d) Manner of seeking (graded 0-4).

0 = no seeking movements.

1 = rapid exaggerated seeking movements of pelvis.
2 = weak irresolute seeking movements.
3 = good determined seeking movements.
4 = very marked uncontrolled movements with penis moving out and back into prepuce.

(e) Pelvic thrust (graded 0-5)

0 = despite contact with vaginal mucous membrane (or artificial vagina) there is no release of the thrust reflex.
1 = mucous membrane contact releases a rapid withdrawal the penis with a simultaneous hop backwards on the hind legs.
2 = no thrust but rapid movements back and forward in vagina, i.e. "friction coitus".
3 = weak body thrust.
4 = good body thrust without back legs leaving the ground.
5 = good body thrust with back legs leaving ground.

(f) Protrusion of penis on thrusting (graded 0-4).

0 = no protrusion.
1 = slight protrusion.
2 = moderate protrusion.
3 = good protrusion.
4 = very extended protrusion.

(g) Body position during seeking.

Describe any unusual signs, e.g. too far back on cow, failure to grip the cow with the forelegs, marked concavity of the lumbar area, excessive bending of hock joints, etc.

This scoring system will define the pattern of the serving behaviour which will be helpful in interpretation in the light of other findings.

10.1.3.2 *Serving capacity*[2]*:* This is a quantitative measure of the behavioural efficiency of a bull. This test was developed in Australia and was widely used in herd fertility control programmes. Studies have shown that the number of services a bull achieves under paddocked conditions is closely related to the number of oestrus cows it serves and the number it will serve two or more times. The serving capacity has been defined as the number of services a bull achieves in a paddock mating period. This can be accurately predicted by counting the number of services a bull completes in a 40-minute yard test.

The method of performing serving capacity test is as follows:

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2 Blocky (1978)

(a) Heifers are restrained in service crates (stanchions), located at about 7 metre intervals (or more) around the perimeter of the yard. A earth yard is less slippery than a concrete yard. A ratio of 4 heifers to five bulls is used. Heifers are generally not in heat and the service crates restrict movement. Obstetrical lubricant placed in the vagina assists in avoiding trauma. Heifers in oestrus can also be used. Each heifer should only be used for two tests (average of 12 services per test) and then replaced.

(b) Bulls are sexually stimulated before the test by allowing them to watch other bulls mounting.

(c) Bulls are then allowed with heifers for 40 minutes and the number of services (not mounts) are counted.

Bulls should be tested within their own age groups. The groups should also have been running together previously, since the introduction of strange bulls into a yard will promote fighting and cause injury. The space between the heifers is also important in the context of social interaction between bulls. Dominant bulls in the group can inhibit smaller individuals if they are too close to each other.

Studies have shown that bulls with a serving capacity of 3 or more have satisfactory fertility while bulls with a serving capacity of 0, 1 or 2 show low first service conception rates.

Serving capacity tests are useful also in detecting bulls with abnormalities of the penis and prepuce, particularly the inability to protrude the penis and deviations of the penis. Arthritis in the back and limbs shows up more clearly during serving capacity tests rather than during an ordinary clinical examination.

10.2 Semen collection

Semen examination of a bull under evaluation is of paramount importance. Semen can be obtained by means of an artificial vagina, or by electro-ejaculation or by the massage technique. But the method of choice is by means of an artificial vagina. It has the advantage of simulating natural service. The ejaculate is more likely to resemble the normal ejaculate compared to other methods. A teaser animal, however is required.

10.2.1 Semen collection with artificial vagina

In this method, semen collection is carried out by inducing the bull to ejaculate into an artificial vagina (AV). More details of the AV and its assembly is given in Chapter 12. This method requires a suitable place to restrain the teaser, with provision of a good floor for a good firm foot holding. A special service bail should be constructed where regular collections are to be made. When collecting semen for diagnostic purposes, it is usual to allow 10 or 15 minutes for a bull to serve, before trying another method. When a group of bulls is being examined, individual bulls should be led to the teaser cow to serve. If a bull does not serve, another bull should be tried, until one serves. This aids in sexually stimulating the group and other bulls will tend to follow.

Optimum conditions must be provided when an artificial vagina is used in order to get the best results. It must have right temperature, pressure and lubrication. Temperature is the most important factor. Warm water should be poured into the artificial vagina (AV) to achieve a final temperature of approximately 45° C (range 42-48° C). Air is blown into the AV until the opening is closed and the lining bulges out forming a cushion. Lubrication of the AV with petroleum jelly, preferably from a tube should be carried out. Excessive lubrication should be avoided, since excess jelly will tend to contaminate the semen sample.

When the bull mounts, the artificial vagina is held at the side of the cow, on level with vulva. The other hand is used to deflect the penis into it. This is done by application of gentle pressure with the finger at the base of the penis where it is covered by the preputial skin. The mucous membranes of the penis should not be touched at any time. It is very easy to apply too much pressure to the base of the penis and cause discomfort to the bull. Another common mistake is to force the artificial vagina down on to the penis. Care should be taken to avoid such practices. The bull should be allowed to seek the AV on his own and the vagina itself should be held relatively stationary. Two or three collections should be made if possible to obtain as representative a sample as possible and to get a better assessment of the pattern of the serving behaviour.

10.2.2 Collection by electro-ejaculation

This was a widely used method in the past. The preputial hairs are clipped, the area is washed (with water only) and dried. A rectal probe with two or more electrodes is used. A graded series of stimulations is given beginning with a weak impulse, followed by a pause and then impulses of increasing strength with pauses in-between. The preferred wave is the *sine* wave. Various systems are recommended by manufacturers of different machines. In general, grade the degree of stimulation according to the muscular reaction of the bull. Slow conditioning of the bull is important.

10.2.3 Collection by manual massage

Massaging of the seminal vesicles and the ampulae of the vas difference has often been used, when the bull is not trained to serve into an artificial vagina. The preputial area is washed with clean water and dried with a cotton swab or cloth. A well lubricated hand is introduced into rectum and the seminal vesicles are located and manipulated gently. Manipulation should be generally towards the pelvic urethra and intermittent pressure is applied by using the pelvic floor as the base and using a firm stroking motion and also by picking up seminal vesicles and "milking" them with a stripping motion or just "kneading" them. Ampulae are then located and stripped towards the pelvic urethra using the pelvic floor as the base. The ampullae are also picked up and squeezed from as far as down towards the edge of the genital fold as possible.

Some massaging movements, stroking towards the pelvic urethra, can then be made involving both the ampullae especially at the most posterior part and seminal vesicles.

In some bulls, it has been found useful to pick up the area of convergence of the seminal vesicles and ampullae and applying pressure to that area. The prostrate and pelvic urethra are then massaged or squeezed and this elicits strong rhythmic contractions of the pelvic urethra.

Semen is collected into a test tube attached to a funnel, surrounded by a warm water jacket. Note that semen should not be allowed to run down the preputial hairs or drop on to cold glass. Close attention to these details will help to obtain good results with respect to motility of sperms. The first fluid obtained usually consists of a clear accessory gland secretion and this is followed by a more sperm-rich fraction. It is possible to obtain repeated samples with similar fractions within a few minutes after the first collection.

10.2.4 Collection from the vagina of a cow

This is the oldest method, which still could be used, if other methods are not available or successful. In this method, it is a good idea to clean out as much mucus from the cow's vagina as possible before the bull is permitted to serve the cow. The semen is extracted from the anterior part of the vagina with the hand. Washing with a dilute solution of sodium bicarbonate is recommended as a suitable antiseptic for this purpose.

10.2.5 Advantages and limitations of different collection methods

The above-mentioned methods have their own advantages and limitations. The advantages of AV method are two fold. It allows the bull to serve naturally and it is presumed that the ejaculate obtained is more like that delivered to the cow, than ejaculates collected by other methods. Secondly, it allows the serving behaviour to be assessed. However, this method has disadvantages too. It requires the availability of a cow in heat. The operator has to come into close contact with the untrained bull, that may be a little risky. Furthermore, if the bull does not serve, it is difficult to determine whether or not the bull is incapable of serving or whether it does not want serve into an AV. It is also necessary sometimes to wait for a considerable length of time before one can decide whether or not the bull will serve.

Electro-ejaculation proves to be the easiest method for use on untrained bulls. It does not require a cow in heat, but the presence of such a cow does increase the quality of the samples. The restraint required is fairly simple for handling bulls and a crush is adequate for trained bulls. It is advisable to place a wooden plank behind the hind legs of the bull to prevent the bull from slipping, as it stretches out during stimulation. Another advantage is that many bulls can be examined, at 10-20 min. intervals, provided laboratory facilities are available to carry out the semen evaluation. However, many argue that this is not a very humane method as the animal is subjected to vigorous muscular contractions. Moreover, one cannot be certain whether a bad sample is the result of machine malfunction or whether it is attributable to the bull or the operator. A further disadvantage is the high cost of the machine.

The massage method is the best method in terms of cost and convenience. It requires

only a glove and a collecting vessel. If a cow in heat is available, the process becomes easier and the quality of the sample would be better. Restraint is very simple for handled bulls and a crush is adequate for unhandled bulls. Again, up to six bulls an hour can be assessed by this method. Another advantage is that rectal examination of the bull is automatically done and so a full clinical examination can be completed at the time of collection of semen. The disadvantage is that it causes fatigue to the arm of the operator. There is also the problem of determining whether a bad sample could be attributed to the bull, the failure of applying a correct massaging technique or problems associated with the semen collection technique.

The recommendation is that the use of an artificial vagina, is the method of choice. Where this is not practicable, the massage method is recommended. If a suitable sample is not obtained after two or three attempts, the electro-ejaculator should be used. The last choice may be to collect semen directly from the vagina of the cow. Although this is a very easy method to employ, the semen often becomes contaminated with vaginal secretions and it also involves an additional step between ejaculation and examination.

10.3 Semen evaluation methods

Semen evaluation is one of the important aspect of bull evaluation for breeding soundness. Semen evaluation is also done on a routine basis for bulls used in artificial insemination programmes. Besides, semen evaluation is carried out in infertility investigations to confirm or eliminate the bull factor. In time to come, semen evaluation may become a routine procedure prior to the sale of bulls for breeding, as it has been the case in developed countries.

Semen evaluation consists of two broader steps: (1) the initial examination of semen that involves macroscopic evaluation of volume, colour and density and (2) the detailed examination of semen for concentration, percentage of live and dead sperms (live:dead ratio), percentage of morphological abnormalities of the head, mid and tail and also for the presence of cellular elements other than spermatozoa.

10.3.1 Initial evaluation

A semen sample following collection is subjected to examination for determination of (a) volume (ml), (b) colour (whitish, creamy, yellowish, etc.), (c) density (watery to creamy in a scale of 0 to 6 scale) and (d) motility (wave pattern and percentage of motile sperms).

10.3.1.1 *Volume:* Volume is usually read directly from the graduated collection tube. The volume could range from 1.0 ml to 15.0 ml.

10.3.1.2 *Colour:* Colour of semen could be assessed with the naked eye. It could range from whitish through creamy to yellowish. If blood is present, this would be reflected directly by the colour.

10.3.1.3 *Density:* Density could also be assessed with the naked eye. A grading from

watery to creamy is given on a scale of 0 to 6. (0= watery, 1= thin milky, 2 = moderately milky, 4 = milky, 5 = creamy, 6 = thick creamy).

10.3.1.4 *Motility:* Motility could be expressed in two ways; in terms of wave motion and percentage motility. To determine wave motion, place a drop of undiluted semen on a glass slide and examine it under the low power of the microscope (x 100) to assess the intensity of wave motion and swirl. Give a grading on scale of 0 (no wave motion) to 6 (distinct wave motion). Individual motility and hence the percentage motility is assessed by examining a drop of semen, covered with a cover slip under high power (x 400) of the microscope. In this examination, care should be taken to differentiate sperms that are actively moving forward from those which are being carried along as "passengers". A warm stage is needed to standardise the temperature of the semen at 37°C for both of the above examinations.

10.3.2 Detailed semen evaluation

The semen sample must be subjected to detailed examination in a laboratory. This is done usually at the AI centre before the animal is selected as a semen donor and when the non-return rate is higher than the norm. Field Veterinarians could also send a sample for detailed evaluation to the Semen Evaluation Laboratory at the Department of Veterinary Clinical Studies of University of Peradeniya. For the purpose of making such requests, veterinarians should collect a standard packet containing slides, preservatives and buffers.

Standard procedure for detailed semen evaluation.

10.3.2.1 *Concentration of sperms:* The concentration of sperms can be assessed by using a standard Haemotocytometer or the counting machine (e.g. photometer). The method using the Burker's chamber is given below.

Procedure: Prepare a diluted semen sample with 0.1ml of semen in 9.9 ml of distilled water. Shake the solution well and place a drop of the solution on the edge of the haemotocytometer. Examine under the light or phase contrast microscope (x 400) and count sperms within the specified number of squares (usually 25 squares) excluding the sperms found on two sides of each square (shaded border in Fig. 10.1). Using the formula given below calculate the sperm concentration.

Volume of a small square = 1/250 mm^3
Dilution rate = S
Number of squares counted = A (usually 25 are counted)
Number of sperms in A squares = N
Concentration = N x S x 250/A

10.3.2.2 *Live to dead ratio:* To estimate the live to dead ratio, 3 to 5 drops of semen (diluted or undiluted) are added to warm nigrosin-eosin solution (0.5 to 2 ml) and mixed gently. The mixture is then allowed to stand for three minutes at 37°C. Several thin smears on warm slides are made with the solution in the standard manner. A drop

of solution is placed on the edge of a slide and a second slide is held at an angle of 45°C and dragged over the slide to make a thin smear either by forward or backward movement. The smear is then examined under the microscope (x 400). The dead sperms take up the dye (eosin) and are stained pink, while the live sperms appear colourless as it exudes the dye. The nigrosin stains the background. Count at least 200 sperms and record the number of dead (pink sperm) and the number of live (colourless sperms). Express the results as a ratio of live to dead sperms.

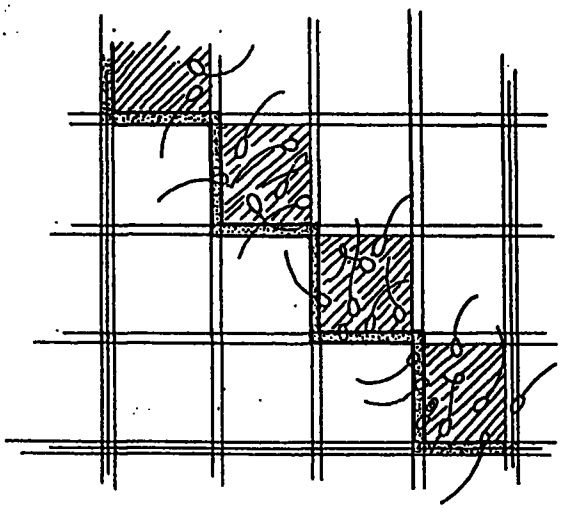

Fig. 10.1 Semen counting using Burker's chamber

10.3.2.3 *Head morphology:* Head morphology is examined by staining a semen smear with William's stain (Carbol-fuchsin eosin stain). Prepare a thin smear with fresh semen as described above. Dry the smear and fix over a flame and then stain with William's stain as described below.

Procedure: A dried and fixed smear is treated in the following manner: (1) dip in absolute alcohol for 3-4 minutes and dry in air, (2) dip in 0.5% Chloramin - T solution for 1-2 minutes until the mucus is removed and the smear appears fairly clear, (3) wash in distilled water and then in 95% alcohol, (4) dip in carbol-fuchsin solution for 8-10 minutes and (5) wash in water, dry the smear and examine under the phase contrast or light microscope. Alternatively, the smear could also be made from the semen mixed with Nigrosin-eosin solution as described earlier.

Count 500 sperms and look for normal and abnormal head morphologies. The types of sperm head morphologies that may be encountered are given in Fig. 10.2.

10.3.2.4 *Mid and tail morphologies:* A buffered formal-saline solution is used to prepare the semen for a count of abnormalities associated with the mid and tail of sperm as described below.

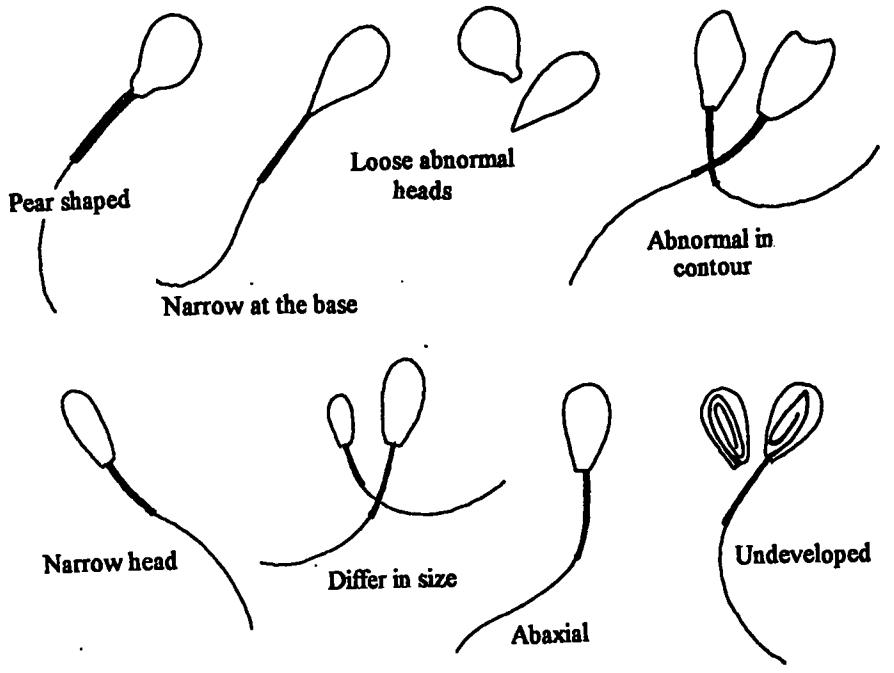

Fig. 10.2 Sperm head abnormalities

Procedure: A few drops (2-3drops) of fresh semen are added to a vial containing formal-saline (approx. 2 ml) and mixed gently. This will fix the semen sample permanently. A drop of this solution is then examined under the high power (x 400 or 1000) phase contrast. Record the various types or forms of head and tail of sperms. Count 200 sperms. Categorise the abnormalities in the form shown in Fig. 10.3 below. When phase contrast is not available, the same assessment can be made on a nigrosin-eosin stained smear. The count is then slightly less reliable.

10.3.2.5 *Cells other than spermatozoa:* To estimate the presence of cells other than spermatozoa, such as leukocytes, erythrocytes, epithelial cells, medusa cells and spermatogenic epithelium, prepare a thin, but intermittent smear of semen. Place a drop of semen near the end of one slide. Place a second slide over the semen held at an angle of 45°C and draw the semen over the slide by a backward movement, but lift several times during the stroke leaving bands of thicker semen at intervals along the slide. Dry the smear and stain with Haematoxylin-eosin stain in the standard manner.

Procedure: The dried smears are treated in the following manner: (1) dip in absolute alcohol for 3 minutes, (2) dip in 95% alcohol for 3 minutes, (3) dip in 70% alcohol for 3 minutes, (4) dip in 35% alcohol (for washing), (5) dip in haematoxycilin stain for 10 minutes, (6) wash with tap water, (7) one dip in 1% HCL in 70% alcohol (to take away excess stain), (8) keep under a running tap for 5 minutes, (9) dip in eosin

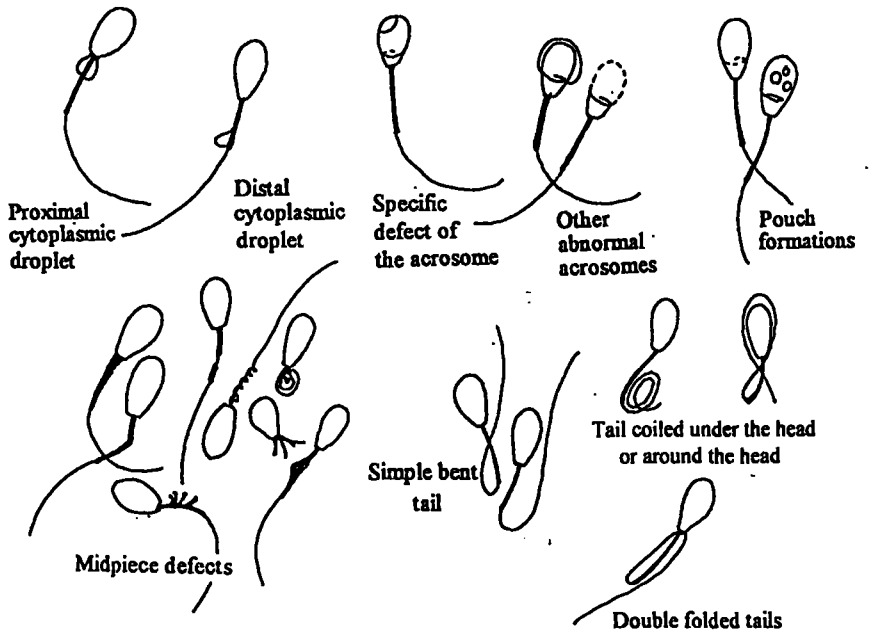

Fig. 10.3 Mid-piece and tail abnormalities

stain for 5 minutes, (10) dip in 70% alcohol wash, (11) dip in 95% alcohol wash, (12) dip twice in absolute alcohol, wash, (13) dip in Xylol for 2-3 minutes and (14) examine the smear under the low power (x100) of microscope. Record the number of abnormal cells other than sperms (Fig. 10.4).

Routine results of semen examination can conventionally be listed as in (Table 10.1) to give a complete view of ejaculate characteristics. For reference purposes, the semen values consistent with normal reproduction are also listed. Careful consideration, however must be given to the source of variation from the normal range. Variation could be due to the bull or the operator or the technique used.

For example, the volume will vary according to the amount of fluid ejected by the bull and the amount collected by the operator. Density and concentration will depend upon the efficiency of the contraction of the vas deference and epididymal tail in response to stimulation (e.g. cow in heat, massage, electrical, etc.). Motility can be low when the bull has not served for a period of time, when the sperms have been cold shocked, when there is water or soap in the collection vessel or when the rubber of the artificial vagina is toxic to sperms. With massage or electrically stimulated samples, motility is sometimes low for unexplained reasons associated with the technique. The percentage will be low if the details of technique were not strictly adhered to. The percentage of

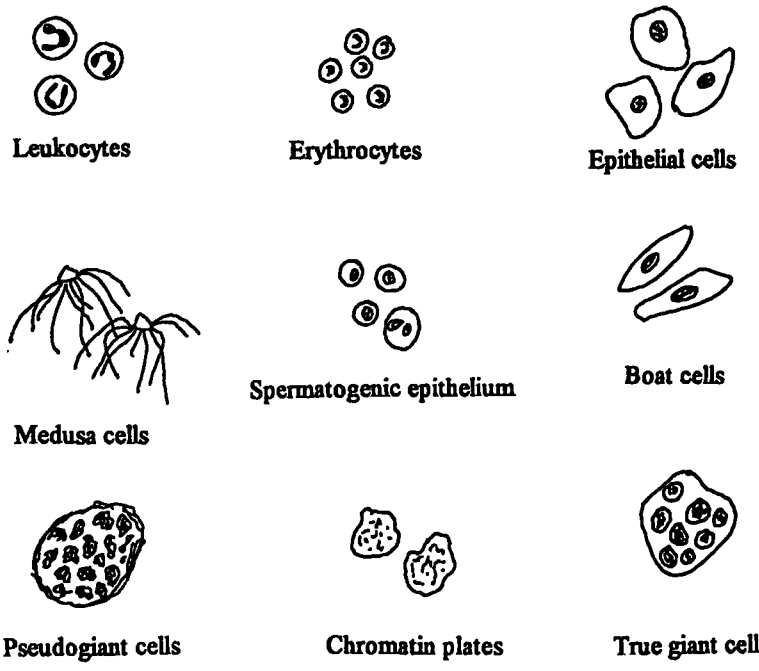

Fig. 10.4 Cells other than spermatozoa

sperm with droplets attached will be lowered if the sample is not preserved immediately after collection. Droplets become detached with time of agitation. The percentage of tailless heads may increase with lapse of time and with agitation. The percentage of singly bent tails may be increased by cold shock or osmotic damage (water in the tube). Leukocytes present may be from the lower or upper parts of the genital tract. The method of collection will have some bearing on the number found. For example, they may be from the prepuce when there is inflammation present.

10.4 Test for infectious diseases

Bulls used for breeding, whether natural or artificial, must be free of any diseases which could be transmitted to other animals through coitus or insemination. The diseases of importance are brucellosis, tuberculosis, leptospirosis, rinderpest and foot and mouth disease. Animals with these diseases must be examined using standard isolation and serological techniques.

10.5 Cytogenetic examination

Chromosomal examination plays a very important role in fertility evaluation. It has been well demonstrated that certain chromosomal abnormalities are associated with infertility or sometimes sterility. This particularly poses a problem if a bull with such abnormality is used for artificial breeding. Therefore in almost all developed countries, bulls are evaluated using cytogenetic techniques before they join a semen donor programme.

Table 10.1 Semen parameters consistent with normal reproduction

	Values of semen parameters
Volume (ml)	1-15
Density (0-5)	3
Wave motion (0-6)	3
Motility (1-100%)	50
Concentration (per mm3)	500
% sperm alive	>50
% abnormal heads	<20
% abnormal mid pieces	<2
% sperm with proximal cytoplasmic droplets	<4
% sperm with distal cytoplasmic droplets	<4
% sperm with tailless heads	<15
% sperms with singly bent tails	<8
% sperm with double bent tails	<4
% sperms with coiled tails	3
Cellular elements other than spermatozoa	None or very few neutrophils and epithelial cells

Source: Galloway (1983).

Selected references

Blockey, M. A. de B. (1978) Influence of serving capacity on bulls on herd fertility. *J. Animal Science*, **45**, 589-595.

Cole, H. H. and Cupps, P. T. (1977) *Reproduction in Domestic Animals*. (Third Edition). Academic Press, New York, USA.

Galloway. D. B. (1983) *Breeding soundness examination in the bull*. Melbourne University, Australia.

Peters, A. R. and Ball, P. J. H. (1995) *Reproduction in Cattle*. (Second Edition). Blackwell Science Ltd., Oxford, UK.

CHAPTER 11

Artificial Insemination in Cattle and Buffaloes

H. Abeygunawardena

Introduction

Artificial insemination (AI) is the most widely used biotechnology in the world. The first significant development in practical application occurred at the beginning of this century with the discovery of semen collection methods for cattle and other farm animal species in Russia and Denmark. Since then, it has expanded and spread across the world and the technique was first introduced into Sri Lanka in 1937. AI was first carried out on an experimental basis at the Meewatura Farm and later AI was introduced on a limited scale to farmers around Peradeniya. But the acceptance by the farmers was low. However, with the dawn of independence, the value of AI in genetic improvement programmes was realised and AI services were expanded into more areas with the establishment of the Semen Collecting Centre at the Veterinary Laboratory at Peradeniya, which was subsequently transferred to Kundasale (Central Artificial Insemination Centre - CAIC). Semen processing centres were later established, one at Tinnaveli and another at Polonnaruwa. Deep frozen semen technology was introduced into the country in 1957. At present, only deep frozen semen is used in the country.

The progress of the AI service, however has been hampered due to problems emanating from poor infrastructural facilities, indifference and lack of awareness among farmers, poor technical expertise of technical staff and problems associated with cows. According to statistics relating to the mid 1990s, less than 20% of the breedable cattle and less than 1% of breedable buffaloes were bred through AI. Of these, according recorded calvings, only about 25% of AIs resulted in pregnancies. Nevertheless, the AI service has shown a steady progress in the extent of coverage, number of inseminations performed and also calving rates.

The success and factors limiting the successful operation of AI services have been studied by many workers[1,2]. These studies have highlighted that the success rate in terms of conception rates was lower than the acceptable level and this is a result of many factors emanating from the farmer, cow, semen and the technician. These studies highlighted the need for improvement in all aspects, from semen processing to insemination and most importantly the skills of the farmer and the technician.

The purpose of this Chapter is to provide a very short description with regard to (1) semen collection (2) processing, (3) storage, (4) technique of insemination and (5) monitoring and evaluation of AI programmes.

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1 Alexander *et al.* 1997

2 Alexander *et al.* 1998

11.1 Selection of bulls as semen donors

Bulls which are of high genetic potential are selected as semen donors. The process of selection is discussed in Chapter 10. Once they are selected, they must be subjected to rigorous testing for desirable characters - gait, posture and the strength of hindquarters. They must also be tested for freedom from several disease conditions such as Tuberculosis, Leptospirosis, Camphylobacteriosis, Babesiosis, Trichomoniasis, Infectious Bovine Rhinotracheitis (IBR), Enzootic bovine leucosis and Bovine viral diarrhoea. They must be free of all the above disease conditions and others which may be important. After the initial testing, the donor bulls must be tested annually to ensure that they remain disease free.

11.1.1 Semen collection

The standard procedure for collection of semen is with the use of an artificial vagina (Fig. 11.1). The AV must be well lubricated and the internal temperature must be maintained between 42°C - 48° C. The length of AV should be chosen so that the bull will ejaculate directly into the funnel, in order to avoid sperm loss. If more than one ejaculate is to be collected, a separate AV should be used for each collection.

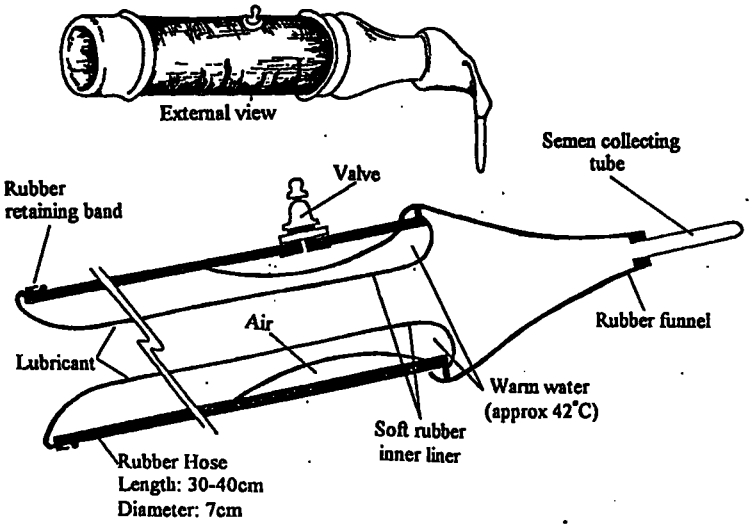

Fig. 11.1 Artificial vagina

To maximise the sperm output, it is important to sexually stimulate the bull before ejaculation takes place. This is accomplished by means of false mounts, restraining the bull as it attempts to mount or by letting other bulls come near to or mount the teaser animal. Steers are preferred as teaser animals, to prevent vaginal intromission and possible transmission of diseases.

The frequency of collection should be arranged according to the capacity of the individual bull. The normal schedule is to collect one ejaculate twice a week, but mature bulls may be used three times a week. Two subsequent collections may be

taken from certain bulls each day in order to increase the sperm harvest. Young bulls may be used at intervals of 7-14 days. It is through experience that one can decide whether an individual bull can be used for two, three or more collections weekly. Other necessary conditions for a better harvest are clean surroundings, good floor and the use of crates. Motivation and skills of people handling semen donors is also an important factor.

11.2 Semen evaluation

Semen samples collected must be subjected to rigorous laboratory testing, before processing. A number of semen characteristics can be assessed or measured to ensure that only semen of good quality is used for processing. The true fertilising capacity can, however be evaluated only by calculating the pregnancy rate following insemination with semen. Years of experience nevertheless have shown that there exists a good correlation between the breeding efficiency and the semen parameters estimated in the laboratory. A detailed description of macroscopic evaluation, microscopic evaluation, concentration estimation and morphological evaluation is given in Chapter 10.

11.2.1 Macroscopic evaluation

11.2.1.1 *Colour*: Colour of bull semen could range from creamy, white or grayish. But sometimes, semen from some sires could be yellow and it is usually due to the presence of pigments, either lipochrome from the ampullae or a flavin from the prostrate. Pink or red colour may indicate contamination with blood or dirt. The presence of blood could usually be due to mechanical injury to the glans penis or urethra. A brownish colour in semen may indicate decomposed blood originating from disease conditions in the deeper part of genital tract or organs. The presence of pus indicates, an inflammatory process somewhere in the genital organs.

11.2.1.2 Volume is directly measured from the graduated markings of the collection tube. Volume in general could range from 1 to 15 ml, depending on breed, age, method and frequency of collection. Variations in volume in a routine schedule of a particular bull may indicate either a disease condition or improper collection procedure.

11.2.1.3 *Density* of semen is a crude measure of concentration of sperms. It may be described as watery (low concentration of semen), milky (moderate concentration of sperms) or milky (high concentration of sperms).

11.2.2 Microscopic examination

There are a number of microscopic characteristics that could be assessed in the laboratory on a routine basis. But the most commonly performed tests are (a) determination of the wave pattern or mass activity, (b) motility evaluation and (c) the live : dead ratio.

11.2.2.1 *Mass activity* refers to wave motion of live sperms and this is of prime importance as the first criterion which indicates whether an ejaculate is suitable for further processing or not. Mass activity is examined in a droplet placed on a glass slide without a cover slip, placed on a warm stage and viewed at a magnification of 80 or 100. Usually mass activity is assessed on a scale of 0 to 6 as discussed in Chapter 10. Usually semen samples above 3 are accepted for further processing. However, note that different laboratories have their own criteria in selecting semen samples for processing.

11.2.2.2 *Motility* is of an important criterion in ensuring successful conception. It is assessed by examining a diluted sample (usually 1:20) in a droplet preparation under a cover slip on a warm stage, usually at a magnification of 200. Motility is rated as the percentage of motile spermatozoa showing progressive forward movement while sperms that are oscillating, or exhibit either circular or backward movements are not included. Usually motility is rated as a percentage and semen samples showing less than 70% are not considered for processing (note: these standards may change between laboratories).

11.2.2.3 *Live:dead ratio*: This test is not routinely performed in many laboratories, but when an abnormality is suspected it could be a very rewarding test to perform. As described in Chapter 10, it is done by staining a smear made from a mixture of fresh semen and Nigrosin-eosin staining solution. The percentage of live spermatozoa must be above 50% to consider the semen as suitable for processing.

11.3 Morphological evaluation

Apart from above-mentioned routine tests, periodic evaluation of semen samples for morphological parameters must be performed. This is always done at the time of selection of bulls for AI programmes. Besides, these tests become necessary when a semen sample deviates from the normal range in terms of motility or percentage of live sperm. In general, all bulls in an AI programme must be checked for morphological parameters of semen on a yearly basis. The standard tests as described in Chapter 10 are: (a) head morphology evaluation using William's stain, (b) mid-piece and tail abnormalities using formal-saline fixed samples and (c) examining intermittent smears with haematoxylin-eosin for cells other than spermatozoa. The detail procedures are also described in Chapter 10.

A certain number of sperm abnormalities can always be found in semen from normal bulls. But the presence of a considerable number of sperm abnormalities should always draw attention to the possibility of lesions being present in the spermatogenic epithelium, such as orchitis, testicular degeneration, fibrosis, or testicular hypoplasia.

11.4 Semen processing

It has long been recognised that the fertilising capacity of semen could be preserved for an extended period by mixing semen with suitable media (extenders), depending on the method of storage. Two types of storage methods are used in practice: (a)

chilled semen, where semen is preserved at + 4 to 5° C and the fertilising capacity will last for a few days and (b) frozen semen where semen is stored at – 196° C and the fertilising ability lasts for several years. The constituents of typical extenders and the functions of each constituent used in chilled and deep frozen semen are given in Table 11.1.

Table 11.1 Functions and constituents of extenders used in semen preservation

Function	Constituent	
	Chilled semen	DF semen
Vehicle	Skimmed milk	Skimmed milk
Cryoprotectant		
cooling	Egg yolk	Egg yolk
freezing	----------	Glycerol
Energy source	Fructose	Fructose
Antibiotics	Penicillin/Streptomycin	Penicillin/Streptomycin

Source: Peters and Ball (1995)

11.4.1 Chilled semen

The viability of sperms could be extended at + 3 - 5°C for 3 - 4 days when semen is mixed with buffers or extenders. The commonly used extenders are egg yolk-phosphate or egg-yolk-citrate. Liquid semen is processed by diluting the semen sample in two stages using egg-yolk based diluents at the temperature of around 17° C. Besides the egg-yolk-based extenders, there are a number of other extenders in use such as skim milk extenders, Illinois variable temperature diluent (IVT), TRIS extender, etc. Usually all extenders are fortified with antimicrobials, such as penicillin (500-1000 IU/ml) and streptomycin (0.5 mg/ml) routinely. Processing of liquid semen is less rigorous and the loss of sperm is less than 10% compared to sperm losses during stressful freezing and thawing in the frozen semen technology.

Chilled semen is usually available in vials containing several doses of semen. In some countries, it is available in straws. While chilled semen has the inherent problem of a very short shelf-life, it offers certain advantages such as: (1) the number sperms per unit volume (sperm dose) is low compared to deep frozen (DF) semen, which allows much better utilisation of semen, (2) approximately 2-5% higher fertility when compared to DF semen, (3) the processing technique is less sophisticated and (4) less care is required in handling semen under field conditions compared to DF semen.

11.4.2 Deep frozen semen

The viability and fertilising capacity could be extended for years by storing at -196°C. This technology became feasible only following the accidental discovery of the ability of glycerol to protect sperms during freezing. It prevents the rupture of the cell wall during freezing.

In this process, semen is diluted over 5 stages in either skim milk or egg-yolk-based diluent or extenders, with glycerol as a cryoprotectant. A diluent (extender, fructose,

to provide energy, antibiotic and glycerol at the rate of 3 - 10%) is added to semen to achieve a concentration of 60-80 million sperms per millilitre. The diluted semen is then cooled to +4°C over several hours before introduction into ampoules (1 ml), straws (0.25 ml) or pellets (0.1ml). In Sri Lanka straws are used to store DF semen. The straws are then sealed and placed in a freezing rack in a computer controlled freezing chamber. The frozen semen straws are then packed into goblets and are stored in liquid nitrogen at a temperature of -196°C until required for insemination.

This technology has made it possible for implementation of breeding programmes which are based on better utilisation of outstanding bulls and on more rational planning of the daily work at semen banks. Successful inseminations have been performed with DF semen stored for over 20 years. However, it has its own disadvantages as freezing and thawing could damage sperms. It has been estimated that 30 - 40% of sperms are killed during the freezing and thawing process. Hence, a larger number of sperms per dose of semen has to be incorporated. Fertility following the use of DF semen is 2-5% lower than that achieved with chilled semen.

Handling of DF semen, however requires special care. Transfers between containers should be kept to a minimum, as the risk of damage is greater with increased handling. Frozen semen should not be exposed to air for more than 2 seconds during the transfer. Semen starts thawing at -130° C. The recommended temperature for thawing is between + 32 to 37° C and any equipment that comes into contact with thawed semen should be kept warm to prevent cell damage.

11.5 Storage of deep frozen semen

Deep frozen semen is stored in liquid nitrogen at -196°C. It must be noted that liquid nitrogen is dangerous and must be handled with care. Safety instructions are given in the Appendix to this chapter. The diagrammatic description of a typical liquid nitrogen storage tank is given in the Fig. 11.2a. Semen straws packed in goblets are stored in canisters (long and short canisters). The liquid nitrogen must cover the straws and fill the goblets while the semen is stored in the tank. Semen straws may maintain fertility when stored in liquid nitrogen vapour, but two problems may arise if the liquid nitrogen level is allowed to drop to very low level.

Straws will be more susceptible to 'temperature' damage if they are lifted into the neck of the tank when liquid nitrogen is not present in the goblet. As shown in Fig. 11.2b, the temperature increases rapidly towards the top of the neck of the tank. The other problem is that nearly empty goblets may float out of canisters when the tank is refilled with liquid nitrogen.

As stated earlier, sperms are fragile and therefore must be handled in the right way to achieve the best results. Hence, maintaining at least a minimum level of nitrogen in the storage tank is essential and most importantly the straws must not be exposed to outside temperature for more than 2 seconds. Further, exposure to direct sunlight, dust, water, soap, powder or rough handling may reduce conception rates. It is very

important that identification of the semen straw is made before removing the straws from the neck of the tank.

11.6 Equipment required for artificial insemination

These are the liquid nitrogen cans, the canisters, goblets, straws, the loading gun and disposable sheaths, gloves, paper towels, the thaw box and lubricant. Canisters are the

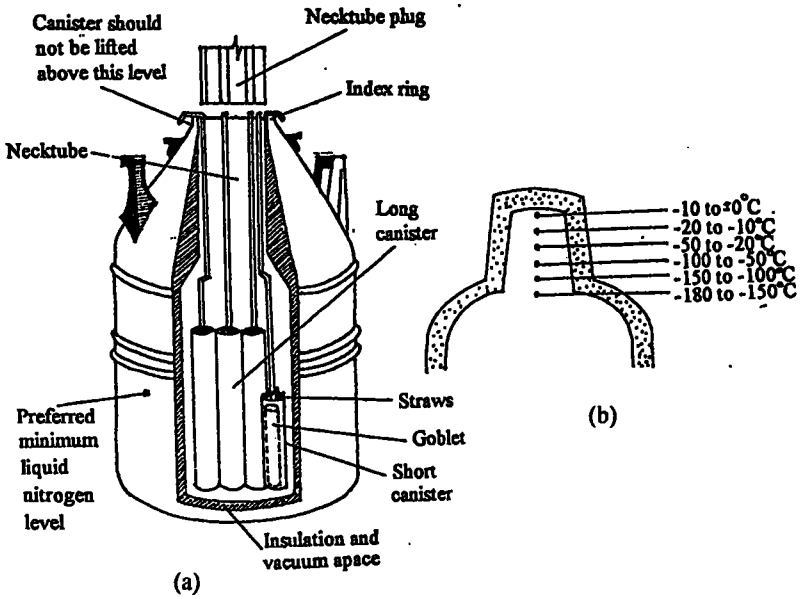

Fig. 11.2 Typical liquid nitrogen storage tank (a) and temperature variation at the neck of the tank (b)

removable cylinders with a wire mesh or solid bottom to hold goblets in the tank. Goblets are the plastic cylinders with a sealed base which fits into the canister and which could hold 25 straws in a bath of liquid nitrogen. Straws (med-straw - 0.5 ml or mini-straw - 0.25 ml), loading gun and disposable sheaths are shown in Fig 11.3 and Fig.11.4.

11.7 Technique of insemination

In order to obtain the full benefit of this valuable technology, cows in oestrus must be inseminated at the correct time and the technique must be carried out with strict adherence to the recommended procedure.

The attending veterinarian and technician must be fully aware that the sperms are very fragile and must be handled with utmost care. Moreover, he or she must be aware that in circumstances where this technique is performed poorly, pathogenic organisms could be introduced into the cow's uterus and the resulting uterine infections will definitely affect the viability of gametes, the embryo and also the implantation process. Hence, it is very important to prevent exposure of the processed semen to temperatures

beyond the recommended temperature limits and to handle the semen preparation (chilled or frozen) in a manner that will keep semen away from contamination with irritants, dirt and other pollutants. To maintain the effectiveness of the technique, precautions must be taken to ensure that contaminants are not introduced into the uterus of cows.

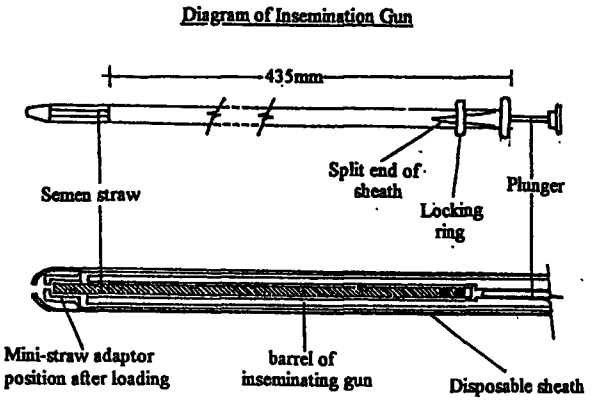

Figure 11.3 Semen straw

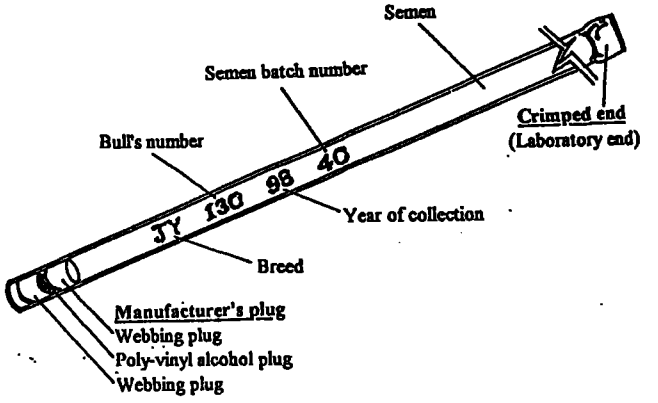

Figure 11.4 Insemination gun

As a guideline to achieve the highest success rate from AI, a hygiene checklist is given below, to ensure the following is complied with before and during insemination:

(i) Always wear new plastic gloves when performing an AI.
(ii) Always use new protective sheaths over the AI gun.
(iii) Use a fresh paper towel or clean piece of cloth.
(iv) Keep the 'cow end' of loaded gun uncontaminated.
(v) Clean the perenium region as much as possible and wipe it completely.

(vi) Introduce the point of the AI gun cleanly as deep as possible into the vaginal vault through the wide open vulval lips.

(vii) Avoid inseminating too deep and causing excessive movements of the gun inside the cow.

(viii) Dispose of the used sheaths, plastic gloves and paper towels properly, and thoroughly clean contaminated guns before returning them to the kit box

(ix) Scrub boots and clean the hands by washing thoroughly before leaving the farm.

11.7.1 Thawing of semen

(i) Prepare the thawing flask by adjusting the water temperature in the thawing flask to within a range of 32 to 38° C (*It is essential to use a thermometer to check the temperature as thawing semen to above 40°C may cause semen to overheat and 'cook'.*). Ensure that the water is deep enough to cover only about 4/5 of the straw. If the water is too deep and the end of the straw is broken, water may enter the straw and kill the sperm.

(ii) Remove the neck plug from the tank.

(iii) Select the canister which contains the required semen. A knowledge of the precise location of semen will minimise unnecessary handling of straws. Keep a semen location diary with the tank.

(iv) Lift the canister to the top of the frost line in the neck of the tank. The frost line is about 50 mm below the top of the neck. Do not bring the canister up to the top of the neck.

(v) Select the required straw or straws from the goblets using tweezers. Use tweezers to avoid warming the straw and causing frostbite to the fingers. Provided the goblet is full of liquid nitrogen, the canister may be held in the neck of the tank for up to 45 seconds before lowering it to the bottom of the canister.

(vi) Whilst holding the laboratory end of the straw, give the straw a flick as this will dislodge any liquid nitrogen that has accumulated at the manufacturer's end of the straw.

(vii) Place the straw in thawing water as quickly as possible leaving it there for a minimum of 30 seconds. Thawing for a loner period does not cause any harm but the straw should be used within 20 minutes of removal from the neck. Once thawed, straws must not be refrozen.

(vii) Replace the neck plug in the liquid nitrogen tank.

11.7.2 Loading the AI gun

(i) Using the fingers, remove the straw from the thawing flask and dry it with a paper towel/cloth. Drying should be accomplished in the following manner: (a) hold the straw by the laboratory end and draw it through the paper/cloth once, (b) grasp the straw by the manufacturer's end (double wad end) and draw it through the

paper/cloth once more. The straw should be held by the manufacturer's end after drying is completed. Do not dry excessively as the heat build up caused by friction may raise the semen temperature.

(ii) Remove the insemination gun from the kit box.

(iii) Pull back the plunger of the gun about 120 to 180 mm.

(iv) Holding the straw by the manufacturer's end of the straw, to avoid damage to the semen through temperature shock, thread the manufacturer's end into the gun as far as it will go. There is an in-built stop preventing it going in too far.

(v) Hold the loaded gun vertically at eye-level, make a horizontal cut 10 mm above the gun with a pair of scissors, cleaned previously. The cut must be at right angle to the straw so that a perfect seal occurs between the straw and the sheath. After cutting the straw, clean and dry the scissors and return it to its location in the kit box.

(vi) Select a sheath from its protective package and place it over the barrel of the gun. Handle only the split end of sheath keeping the cow end clean.

(vii) Push the sheath through the bevelled centre hole of the locking ring and twist it down on the conical seat of the gun (*The protective sheath prevents contamination of the gun and hold the straw in place during expulsion of the semen. It is important that the seal between the sheath and the gun is secure, to prevent semen from leaking into gun*).

(viii) Press the plunger of the gun until the semen is just visible at the end of the gun (*This reduces the strain of stretching the fingers needed to handle the gun*).

(ix) Hold the loaded AI gun in your mouth or place it across the kit box ensuring that the cow end does not come into contact with any object.

(x) Place the paper towel or cloth in your back pocket.

11.7.3 Inseminating the cow

(i) Wear a protective, preferably a disposable glove and apply a suitable lubricant. Wet the gloved hand by dipping into a bucket of clean water, scooping water up with the hand and letting it run back down the arm. Apply a suitable lubricant on the back of the gloved hand.

(ii) Carry the loaded gun in your mouth ensuring that the cow end does not become contaminated through contact with dirty objects.

(iii) Make the cow aware of your presence as a startled cow may kick.

(iv) Retrieve the paper (cloth) from your pocket.

(v) Protect your hand from contamination with a piece of paper towelling (or piece of cloth), then grasp the cow's tail and lift it aside.

(vi) Using the lubricated back of the gloved hand, smear the lubricant across the cow's anus. Form a cone with the gloved fingers and insert the hand into the rectum. Pause at this stage and encourage the anus to relax by gently revolving your fingers. The wide part of your hand can then be eased in without dragging in the rough dry skin

surrounding the anus. Avoid a rough sudden entry which can bruise the anus and cause pain and stress (note: *stress has been reported to reduce conception rates*).

(vii) Thoroughly clean the vulva of all soap, dung and dirt with piece of paper or cloth used on tail. Use a fresh piece, if the paper is too soiled.

(viii) Press the rectum downwards with wrist of the hand which helps to part the lips of the vulva presenting a clean area for inserting the gun.

(ix) Insert the gun cleanly between the lips of the vulva into the vagina. Take care to ensure that the gun passes along the top of the vagina thus avoiding the bladder.

(x) Gently push the gun through the vagina until it reaches the surface of the cervix which will produce a 'grating' feel (Fig. 11.5).

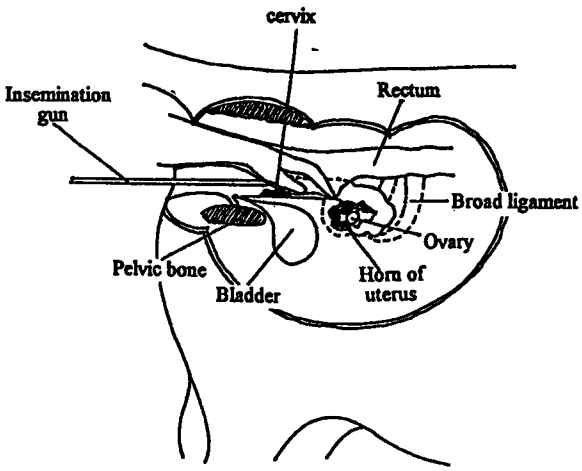

Fig. 11.5 Diagrammatic presentation of the genital tract of a cow and the technique of guiding the pipette into the cervix.

(xi) When the gun reaches the mouth of the cervix, hold the cervix with the finger tips. Maintain a light forward pressure on the gun and manipulate the cervix so that the gun passes through the cervix canal. If you are unable to hold the cervix you may pin it against the pelvis.

(xii) While passing the gun through the cervix, locate the forward end of the cervix with the middle or index finger. Gently push the gun forward but only as far as the locating finger. This is important when doing a repeat service as the cow may be pregnant (*Avoid deep penetration of the uterus as the gun may cause damage and possible infection thus reducing the chance of conception or in the case of pregnant cows, increasing the chance of an abortion. Remember, up to 5% of pregnant cows show some signs of heat*).

(xiii) Begin to express the semen at this position ensuring that most of the semen (two thirds) is expressed in the body of the uterus. Gently express the semen being careful not to 'spit' it out. The remaining one

third should be placed near the uterus in front of the cervix. Take care not to draw the gun too far back, as it is easy to do this at this stage. Occasionally, the gun cannot be passed to the proper position. Avoid bruising and causing other injuries to the reproductive tract by depositing the semen at the position reached after reasonable effort (*Remember, prolonged and forceful struggling will have worse effect on conception rates than incorrect semen placement*).

(xiv) Pause before withdrawing the gun, allowing the semen to get away, then slowly withdraw the gun from the cervix. Rapid withdrawal of the gun can suck semen back through the cervix into the vagina.

(xv) Remove the gun slowly from the vagina and then slowly withdraw the arm from the rectum of the cow.

Following completion of the task, loosen the locking ring on the gun and remove the soiled sheath. The empty straw should come out with the sheath. Dispose of the used straw, sheath, paper and dirty gloves appropriately (e.g. into a waste bag or bin). If the unprotected parts of the insemination gun become soiled with dung or mucus, the gun should be thoroughly cleaned before it is returned to the kit box. Only clean equipment should be replaced in the kit box.

11.8 Monitoring of artificial insemination service

The success of the AI service, not only depends on the quality of semen but also on many other factors. These factors for convenience could be categorised as (a) farm factors, (b) cow factors, (c) semen factors and (d) technician factors. The attending veterinarian must pay close attention to all these points to ensure that the highest success rate could be achieved. Studies carried out by our laboratory have shown that the first service conception rate with AI in the mid-country smallholder production systems was usually around 40%, while the overall conception rate was between of 50 to 55% (see Alexander *et al.*, 1997 and Alexander *et al.*, 1998 for more details). This is in an area with adequate veterinary coverage and supervision. The island-wide averages are much lower. On the basis of the data on reported calvings, the conception rates following AI appear to be in the range of 20 to 30%. The ideal standard considered acceptable in the developed countries for first service conception rates is above 60%, with overall conception rates above 70%. This target is not difficult to achieve. But dedicated efforts of all personnel involved and also of the farmers are of paramount importance to achieve this target. The simplest evaluation process is outlined below. As the more sophisticated methods cannot be implemented at the present time in this country, these will not be recommended.

11.8.1 Maintaining proper records relating to semen

The records that must be maintained are: the date of refilling the tank, dates of arrival of semen, identification of semen along with information on breed, sire, date of processing, etc. Maintain a log book for each with proper identification of semen. This will avoid delays in selecting semen straws and exposure to high temperature. Very often technicians tend to hold the goblet at a point above the recommended level at the

neck of the tank and for an extended period of time.

11.8.2 Displaying information on storage and handling semen

Instructions relating to semen handling during storage must be displayed in the veterinary office. Regularly check the procedures adopted by the technicians in transferring semen straws from the storage tank to the field tank.

11.8.3 Regular examination of semen for quality.

Semen quality must be examined regularly. This could be done with a light microscope. The post-thawing motility must be above 40% to achieve good conception rates. A study done by our laboratory showed that semen quality deteriorates over time and in three months, the motility is well below 40%. Hence, ensure that the oldest semen is used up first, before newer batches are used.

11.8.4 Heat detection

At field level, periodically examine cows reported to be in heat to ensure that farmers are able to detect heat properly and call the technician at the right time. Further, random checks on the procedure adopted by the technician in thawing semen, loading the gun and during insemination should be made by the veterinarian.

11.8.5 Examination of cows not-returning to oestrus

This must be done within 60-90 days post-service by rectal examination to confirm pregnancy.

11.8.6 Examination of repeat breeders

Promptly examine all cows that fail to conceive after three repeated services. Implement necessary corrective measures as described in Chapters 14 and 15.

11.8.7 Monitoring conception rates

Analyse the AI data received from the field on a regular basis and estimate the conception rates achieved. Aim to improve the conception rates to near optimal levels under the prevailing conditions.

Selected references

Abeygunawardena, H. (1999) A review of cattle and buffalo breeding activities in Sri Lanka.. *Sri Lanka Veterinary Journal* **45** (in press)

Alexander, P.A.B.D., Abeygunawardena, H., Perera, B.M.A.O. and Abeygunawardena, I.S. (1997) Current status and factors affecting the success rate of artificial insemination in smallholder farms in mid country wet zone of Sri Lanka. *Tropical Agricultural Research.* 9, 204-216.

Alexander, P.A.B.D., Abeygunawardena, H., Perera, B.M.A.O. and Abeygunawardena, I.S. (1998) Reproductive performance and factors affecting the success rate of artificial insemination of cattle in upcountry multiplier farms. *Tropical Agricultural Research.* **10, 356-371.**

Lewis, I., Owens, J., McClintock, S. and Trevean, M. (1996) *Cattle Breeding Technologies* Genetics, Australia.

Peters, A. R. and Ball, P.J.H. (1995) *Reproduction in Cattle.* (2nd edition). Blackwell Science Ltd., Oxford, UK.

Appendix

Liquid nitrogen must be handled with care. General safety precautions in handling of liquid nitrogen are given below.

1. Make sure that there is adequate ventilation in the storage area. If excessive quantities of nitrogen evaporates in a poorly ventilated room, the percentage of oxygen in the air may become dangerously low.

2. Do not store the tank on a bare concrete floor. Use a wooden plank as a stand for the tank to prevent corrosion. The tank should be kept in a secure place so that children and unauthorized people unaware of the hazards do not have access to it.

3. When handling avoid contact with liquid nitrogen, the liquid or even the cold nitrogen gas issuing from the liquid can cause burns. Brief exposure which may not affect the skin of the face or hands, can still damage delicate tissues such as the eyes.

4. Secure liquid nitrogen tanks in the boot of the car. Carrying the flask in the passenger compartment may be dangerous. In the event of an accident dangerous levels of nitrogen gas will be present in the car if the car windows are shut. Secure the flask at all times when it is being transported. Never use a plug other than that supplied with the tank. The wrong type of plug may interfere with the escape of gas and may cause the tank to bust.

5. Check the tank regularly for evidence of frost on the outside and for excessive loss of liquid. Either of these circumstances could indicate a breakdown of the insulation. The amount of liquid nitrogen in the tank can be detected by the frosting on a dip-stick. Beware of using a hallow tube as a dip-stick as the liquid nitrogen will spray up through the centre.

6. In the event of a skin burn through contact with liquid nitrogen, immediately flood the area with cold water and apply a cold compress (e.g. ice pack). Do not rub the affected area. Seek medical attention immediately. If a person working with liquid nitrogen becomes dizzy or loses consciousness, immediately move person to a well ventilated area. If breathing has stopped, apply artificial respiration and summon an ambulance or seek medical assistance immediately.

CHAPTER 12

Reproductive Management of Cattle and Buffaloes

H. Abeygunawardena

Introduction

The aim of any livestock farming enterprise is to make profits, which depend on output and input costs. By increasing output or reducing input costs, the farmer will be able to increase profits. The extent to which input costs could be reduced depends very much on factors beyond his control, such as market supply and demand. But possibilities to increase output exist as most farm animals presently produce below potential production levels. This stems from poor genetic merit, poor nutrition and poor reproductive management. Reproductive management includes oestrous detection, insemination, pregnancy diagnosis and appropriate and timely interventions as and when required. The latter aspects are dealt with in Chapter 15 as it warrants special emphasis.

12.1. Oestrus detection

Oestrus detection has become an aspect of vital importance in managing cattle and buffaloes in attaining optimum reproductive efficiency. This becomes particularly important as (1) management practices become more intensified, (2) the number of animals maintained per farm holding become smaller and (3) bulls are replaced by the artificial insemination technician.

12.1.1 Heat Detection Methods

12.1.1.1 *Visual observation*: Visual observation by the herdsman or the farmer is the most reliable and also the least expensive method of oestrus detection. Herdsmen may look for behavioural changes in the cow (i.e. departures from routine), physiological changes in the external genital region and other characteristic signs suggestive of oestrus. Standing oestrus may persist for as little as 2 hours with a tendency to manifest more of oestrous signs in the night. There may be at least 15 minute interval between two episodes of mounting. It is therefore very important to observe as frequently and as long as possible for signs of oestrus. It is recommended that in addition to observations at milking and feeding times, three periods of at least 20 minutes are set aside for observation of oestrous signs, at around mid day, during late evening and again around midnight. Proper record keeping will help to reduce the number of days the dairy farmer would be required to set apart for observation of oestrus.

12.1.1.2 *Use of aids in oestrus detection*

(a) *Records*: Farm records are very important for keeping track of all events in the farm as well as those related to individual animals. Unfortunately, record keeping is not practised at all by small holder farmers and not adequately in large state farms. Entries relating to oestrus in individual cow records are particularly important in many

ways.

- Draw attention to cows that have not come into oestrus or display abnormal inter-oestrous intervals
- Help to determine whether the suspected oestrus is genuine by making reference to the previous oestrus date
- Help to predict the next oestrus date and thus reduce the number of days when frequent observations have to be made, as well as increase the chances of detecting the next oestrus

For routine record keeping, a simple field pocket book could be used to record events before they are forgotten. Information from this book then could be transferred to individual record sheets. A wide variety of recording systems have been developed ranging from simple individual record sheets to herd fertility charts and computer-based programmes.

(b) *Heat-mount detectors*: There are various devices that are specifically manufactured for the purpose of detecting oestrus. One such device is the *Kemar heat-mount detector.* This device could be glued to the hair over the rump region, on the midline just in front of the tail head of an animal that is likely come into oestrus soon. Pressure exerted by a mounting cow would squeeze the bag containing the dye and cause the release of the dye from the bag into the reservoir. This change could be easily observed by the herdsman or the farmer when the animal returns from grazing. A cow that had been mounted by another animal is most likely be in oestrus.

Another method is to apply a specially prepared dye as a paste on the midline just in front of the tail head. A smeared patch of dye would indicate that the cow had been ridden by another animal. There is however a danger with both devices as both types of detectors could be accidentally triggered by non-oestrous cows.

(c) *Use of teaser animals:* Teaser animals that will mount on animals in oestrus can be used to draw the attention of the herdsman. Several types of animals could be used as teaser animals.

- A cow in near oestrus or a nymphomaniac cow (i.e. cow with a cystic ovary with high levels of circulating oestrogen).
- A vasectomised bull
- An androgenised steer
- A bull with a surgically modified penis (penectomized or a bull with a deviated penis or a bull fitted with a device to prevent the penis entering the vagina)

(d) *Movement detectors*: A cow in heat tends to become restless. Attempts have been made to measure the movements of the legs (stamping) and correlate these with oestrus. A special device called a pedometer has been used for this purpose.

(e) *Vaginal temperature*: The body temperature of cows tend to drop by one or two points about one or two days before oestrus. This is perhaps due to the withdrawal of

progesterone with the commencement of luteolysis. This drop in temperature is followed subsequently by a short-lived rise in temperature on the day of oestrus. This short-lived peak is associated with the pre-ovulatory LH surge.

(f) *Electrical conductivity of vaginal mucus*: The electrolyte content of vaginal mucosa drastically increases at around oestrus. With the increase in ionic content, there is an increase in efficiency of electrical conductivity as a result of a drop in electrical resistance of vaginal mucus. This has been shown to last 24 hours.

12.1.1.3 *Alternative approaches to heat detection*

(a) *Hormone measurements:* Progesterone measurements either in blood or milk has been used as a method to confirm a suspected oestrus. A low level of progesterone in milk or plasma samples taken on the day of the suspected oestrus would indicate that the cow may be around the time of oestrus. This along with other signs would increase the accuracy of oestrus detection.

(b) *Oestrous control by pharmacological substances:* Oestrus could be induced by using drugs such as prostaglandin $F_{2\alpha}$ or progesterone. The treated cow would come to oestrus within a predictable period of time. This technique is called oestrus induction and synchronisation, which was discussed in detail Chapter 15.

12.2 Optimum time for insemination

In bovines, the life span of the sperm in the female reproductive tract is 18-24 hours and the fertilizable life span after capacitation is only 10 to 12 hours. The female ovulates at varying times after the onset of oestrus. This is because the occurrence of the LH surge in relation to oestrus is not relatively consistent and could be subject to asynchrony of ±8 hours. However, the ovulation consistently occurs between 25 to 30 hours after the LH surge.

Several experiments have attempted to determine the optimum time for insemination for optimum fertility. The results of these experiments shown in Tables 12.1 and 12.2 suggest that fertility could be optimised by inseminating animals from mid heat up to 6 hours after the end of oestrus.

From the data presented in Table 12.2, it is conceivable that best fertility could be achieved by inseminating the animals 12 to 18 hours before ovulation. This would indicate that the best period is usually from about 12 hours after the onset oestrus until about 6 hours after the end of oestrus (from mid-oestrus onwards).

A lag period from insemination to ovulation is essential for the sperm to undergo capacitation in the female reproductive tract before it encounters the freshly ovulated ovum at the ampula of the uterine tube. Studies however, have shown that the sperm could be detected in the ampula region within 5 minutes after the ejaculation. But they are probably not the ones that eventually enter the ovum to effect a successful fertilisation. Further, several studies have shown that there is a transit time between

Table 12.1 Insemination in relation to onset of oestrus

Time of insemination	Fertility (%)
Beginning of oestrus	44.0
Mid oestrus	82.5
End of oestrus	75.0
6 hours after end of oestrus	62.5
12 hours after end of oestrus	32.5
18 hours after end of oestrus	28.0
36 hours after end of oestrus	12.0
48 hours after end of oestrus	0.0

Source: Veterinary Endocrinology and Reproduction (1989)

Table 12.2 Insemination in relation to ovulation

Time of insemination in relation to ovulation	Fertility (%)
>24 hours before ovulation	53.3
19-24 hours before ovulation	73.3
13-18 hours before ovulation	85.7
7-12 hours before ovulation	78.1
< 6 hours before ovulation	57.1
< 2 hours before ovulation	30.0
6 hours after the ovulation	40.0
12 hours after the ovulation	25.0

Source: Veterinary Endocrinology and Reproduction (1989)

ejaculation and the appearance of sperms capable of fertilisation at the ampula and these studies also suggested the existence of sperm reservoirs for sperm transit before they resume pro-ovarian migration. This forward migration is primarily powered by the inherent motility of the sperms and aided by the chemotactants released during the process of ovulation. Studies have also shown that the ovum has a very short fertilisable life span and this is estimated to be as short as 6 - 10 hours.

12.3 Rectal examination and pregnancy diagnosis in cattle and buffaloes

Rectal examination and early diagnosis of pregnancy, forms an integral component of fertility management of cattle and buffaloes. Rectal exploration is a very reliable, accurate and inexpensive method of detecting changes of the reproductive organs (i.e. cervix, uterus and uterine horns, uterine tubes, ovaries, etc.). Examination for pregnancy following an insemination or natural service not only allows detection of pregnancy but also gives an opportunity to detect non-pregnant animals that deserve more attention.

Rectal examination is a relatively simple task for an experienced veterinarian and if performed properly, causes minimum distress to the animal. Further, this technique must be performed in a professional manner. The following description would provide guidelines to perform this task effectively and minimise errors.

12.3.1 General anatomical features and normal disposition of the reproductive organs within the pelvic and abdominal cavities.

The examiner should be very familiar with the anatomy of the pelvic cavity and the associated organs. The reproductive tract (non-gravid) which consists of the cervix uterine body, uterine horns, Fallopian tube (uterine tube) and ovary is located within the pelvic cavity, projecting into the abdominal cavity over the pelvic brim (Fig. 12.1). The entire reproductive tract as shown in Fig. 12.2 is suspended by the broad ligament which is anchored to the abdominal wall. The parts of broad ligament which suspend the uterus, Fallopian tubes and ovaries are called mesometrium, mesosalphinx and mesovarium, respectively.

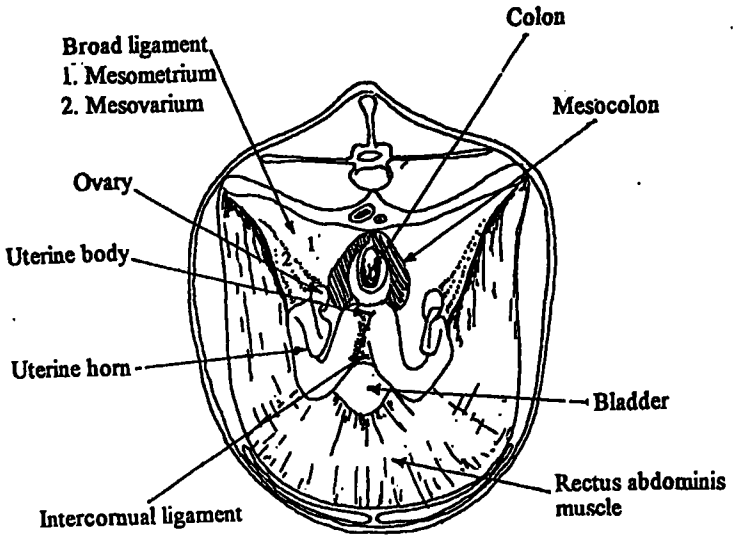

Figure 12.1 General architecture of genital tract (caudal section of the abdominal cavity)

The uterine body and horns are located in between the rectum and bladder and this arrangement makes it possible for the entire reproductive tract to be easily palpated per-rectally. The two uterine horns are held together to some distance by dorsal and ventral intercornual ligaments. The horns initially run forward and then curve (at the greater curvature) and run backward, usually suspended underneath the uterine body. In this arrangement, the ovaries are located just central to or underneath the uterine body and suspended by mesovarium.

12.3.2 Orientation of ovaries and descriptive terminology

As stated previously, the ovaries are usually located in a fossa formed by the broad ligament, and the examiner has to unravel the ovaries from the fossa for detailed examination. The ovary is usually a round or oval structure with transient structures (i.e. follicles and/or corpus luteum), thus giving different shapes. In order to make an accurate examination for recording purposes and also for the use in subsequent

examinations, an assessment must be made of the shape, size (i.e. length, width, height) of the ovary and presence or absence of transient structures (Fig. 12.3).

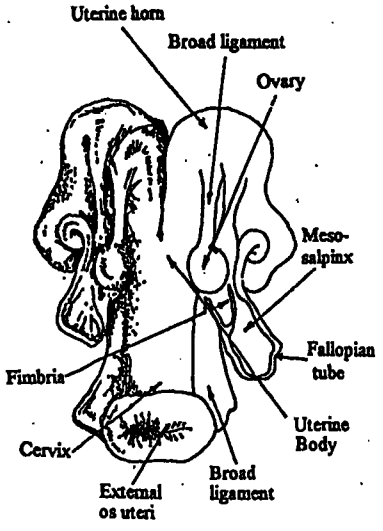

Fig. 12.2 Topography of genital organs

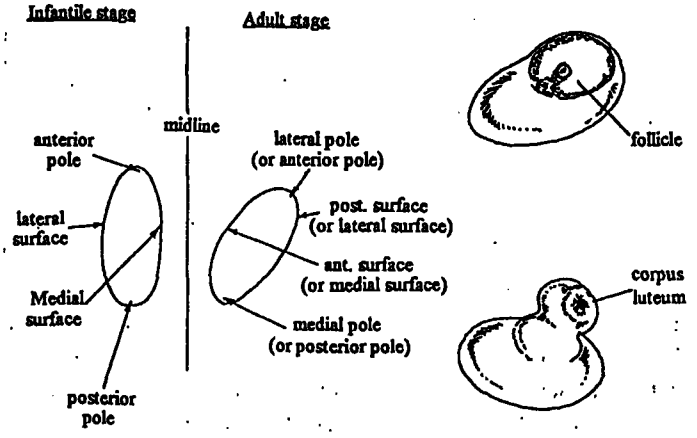

Figure 12.3 Orientation of ovaries and descriptive terminology

As detailed in Fig. 12.3, the dimensions must be given (in centimetres) for, (i) length (from lareral pole to medial pole), (ii) height (from attached border to free border) and (iii) width (from anterior surface to posterior surface).

Note: *In order to use the above terminology to describe the characteristics of ovaries, fix the ovary between index finger and middle finger with mesovarium lying between fingers. The part facing the palm is termed the lateral pole.*

12.3.3 Transient ovarain structures

The ovary is an active glandular structure. Depending on the stage of the oestrous cycle, it could either carry follicles at different development stages and/or a corpus luteum.

12.3.3.1 *Follicles* are fluid filled structures, which appear superficially as slight bulges on the ovarian surface. The surface is smooth but tense and yields to pressure of the finger.

12.3.32 *Corpus luteum* consists of a luteal cell mass with an abundant vascular supply with relatively less connective tissue. It is firm (liver-like and firm) in consistency compared to the ovary proper, which is somewhat relatively harder. The CL usually protrudes from the surface, but in some instances the entire cell mass could be embedded within the ovary. If it protrudes, a well demarcated crown and a neck could be palpated. With age, the consistency changes from soft (developing or early CL) to firm or liver-like consistency (fully developed or mid CL) to hard (regressing CL). Functional categories of an ovary on the basis of the presence or absence of and characteristics of transient structures, the functional status of the ovary could be specified as shown in Box 12.1.

Box 12.1 Grouping of the functional status of the ovary on the basis of the size and the presence or absence of transient structures.

Inactive ovary	-	small ovary, smooth surface devoid of tertiary follicles or corpus luteum
Active or cycling ovary	-	large ovary, bearing a corpus luteum and with or without follicles.
Within the cycling category	early luteal phase -	soft CL tissue (corpus haemorrhagicum or developing CL)
	mid-luteal phase -	CL tissue with liver-like consistency
	late-luteal phase -	CL tissue, somewhat harder in consistency (regressing CL)
Cystic ovary -		large ovary with one or more large follicles (usually the diameter is greater than 2 cm)

12.3.4 Pregnancy related changes

Pregnancy induces changes in the reproductive organs and their associated organ systems, resulting in remarkable changes in the organs.

The following features can be detected *per rectum*, which will enable the veterinarian to determine whether the cow is pregnant or not. Not all of these features are present at all stages of pregnancy.

12.3.4.1 *Thinning of the uterine wall:* The uterine wall of a non-pregnant uterus is approximately 9 to 10 mm in thickness, whereas in the intercaruncular areas of the pregnant uterus, the wall is only about 2 mm thick.

12.3.4.2 *Asymmetry and increase in diameter of the uterine horns:* In the majority of cases, the increase in diameter is more marked in the pregnant horn than in the non-pregnant horn. Remember that pregnancy is not the only state which leads to such an increase in diameter.

12.3.4.3 *Turgidity:* The pressure of the fluid within the uterus is greater than the intra-abdominal pressure in a pregnant animal. Turgidity can be recognised by the fact that, when the hand is passed from side to side over the uterus, it presents a convex surface. This is particularly appreciated within first 3 months of pregnancy.

12.3.4.4 *The amniotic vesicle:* During early pregnancy, the pressure within the amnion is greater than the pressure within the allantois, which in turn is greater than the pressure within the abdomen. At this stage, the amniotic vesicle can be felt as a spherical mass approximately 4 cm in diameter.

12.3.4.5 *Foetal membranes*: These can be detected by the feel of the membrane "slipping" when uterus is grasped through the rectal wall. The grip with the fingers will hold both the uterine wall and also the chorionic membrane. When the grip is relaxed, the membrane (chorion) first slips through the fingers, before the uterine walls slips through.

12.3.4.6 *Placentomes:* These are readily felt as thickenings of the uterine wall. Placentomes comprise the foetal cotyledons and maternal caruncles. They begin to appear in 45 days, but can be palpated per-rectally only after 60-65 days.

12.3.4.7 *Foetus:* The presence of the foetus is the most reliable guide to pregnancy. However, it cannot be felt throughout much of the pregnancy.

12.3.4.8 *Increase in diameter of the middle uterine artery:* The diameter increases particularly on the pregnant side.

12.3.4.9 *Alteration in the character of the pulse in the middle uterine artery*: Alteration in pulsation, particularly on the pregnant side could be detected in pregnancy. This characteristic pulse is known as "fremitus" and is considered to be present only when it is detectable with the lightest possible touch at the point where the artery passes from the pelvis into the abdomen.

Summary of suggestive changes of the reproductive organs and related structures are given in Box 12.2. As pregnancy advances, the whole of the uterus, cervix and ovaries will move forward and down in the abdomen, so that it is not possible to feel the ovary on the pregnant side after approximately four months. However, the ovary on the non-pregnant side can usually be felt up to approximately 7 months. In advanced pregnancy, the cervix is invariably well forward in the abdomen but remember that in some cows it occupies this position even when the animal is not pregnant.

Box 12.2 Summary of suggestive changes of the reproductive organs and associated structures.

Note: Diagnosis of pregnancy by rectal examination will depend on the examiners ability to appreciate the following changes of the uterus.

Shape:	Asymmetry of horns
Size:	Larger than in the normal non-pregnant state
Consistency:	Initially turgid (<30 days), but subsequently becomes soft and the wall becomes thinner.
Presence of uterine contents:	Fluid, foetus, foetal membranes, cotyledons.
Changes of the middle uterine artery:	Hypertrophy and in latter stages "fremitus".
Position:	Within first 3 months - the uterus is within the pelvic cavity with horns extending over the pelvic brim. After 3 months, descent starts. Fluid and foetal parts gravitate towards the extremity. Between 5-7 months, the gravid uterus expands in the horizontal plane. By 7 months, as the space in the horizontal plane is not sufficient, foetus ascends towards to pelvis.

However, it must be remembered that in order to confirm a positive diagnosis of pregnancy, one or more of the following should be detected:

- **Amniotic vesicle**
- **Foetal membrane slip**
- **Placentomes**
- **Foetus**

These are called positive indicators or direct evidences of pregnancy.

12.3.5 Criteria used for assessing the stage of pregnancy in cattle are given in Table 12.3 and in buffaloes in Table 12.4.

Table 12.3 Criteria for assessing the stage of pregnancy in cattle[1] (based on European standards)

Age in days	*Characteristic findings*
19-30 days	*Suggestive information: Turgidity and thinning of uterine wall, well developed corpus luteum on ovary; absence of follicles exceeding 15 mm diameter.*
30 days	*Resilience within the cavity of the larger horn: palpation of turgid kidney bean shaped amniotic vesicle (0.5 x 0.75 cm).*

[1] Bongso, A.R. (1977)

35 days	*Asymmetry of horns begins; pregnant horn 2.5 to 3 cm in diameter; palpation of olive shaped (1 x 1.75 cm) amniotic vesicle; foetal membrane slip begins.*
40 days	*Pregnant horn 4 - 6 cm in diameter; palpation of the plum shaped (2.5 x 4 cm) amniotic vesicle. Foetal membrane slip could be easily detected.*
60 days	*Pregnant horn 6 - 9 cm in diameter. Uterus abdominal, palpable, could be retracted into pelvic cavity. Foetal membrane slip could be easily detected.*
70 days	*Pregnant horn 8 - 12 cm in diameter. Hypertrophy of middle uterine artery begins (0.5 - 0.7 cm), no fremitus; cotyledons (0.5 x 0.75 cm) palpable at the base of pregnant horn; descent begins.*
80 days	*Pregnant horn 10 - 14 cm in diameter; cotyledons palpable (0.5 x 1.0 cm), middle uterine artery hypertrophied (0.5- 0.7 cm), no fremitus.*
90 days	*Pregnant horn approximately double the diameter of non-pregnant horn (12 - 16 cm); uterus in the abdominal cavity, foetus 15 cm long; cotyledons palpable (1.0 x 1.5 cm), middle uterine artery 0.5 - 0.7 cm; fremitus begins.*
100 days	*Pregnant horn 14 - 20 cm in diameter; marked increase in turgidity of the uterus; middle uterine artery 0.6 - 0.8 cm.*
120 days	*Uterus sinks below the pelvic brim and distension is less easy to recognize; foetal fluid gravitates towards the extremities of the cornua; cervix is stretched to lie on pelvic brim; cotyledons (1.5 x 2.5 cm) palpable.*
150 days	*Foetus palpable; the presented extremity of the foetus lies within reach in front and below the pelvic brim; cotyledons 2.0 x 3.0 cm in size; fremitus in middle uterine artery (0.7 - 0.9 cm).*
180 days	*Descent completed; internal ballottement of the foetus and foetal parts possible; cotyledons 2.5 x 4.0 cm; fremitus on pregnancy side only, diameter of middle uterine artery is about 0.7 - 0.9 cm.*
210 days	*Ascent begins; internal ballottement of foetus and foetal parts possible; cotyledons 3.0 x 5.0 cm; middle uterine artery of pregnant side twice the diameter of opposite artery (0.8-1.0 cm); distinct fremitus; tension in cervix at pelvic brim; ovaries not palpable.*

240 days	*Large head of foetus palpable at pelvic brim if presentation is anterior; cotyledons 4.0 x 6.0 cm in size; fremitus in both middle uterine arteries.*
270 days	*Head of foetus in pelvic cavity usually; cotyledons 4.0 x 8.0 cm; hyperthrophied middle uterine artery is 1.4 - 1.6 cm in size.*

Table 12.4 Criteria for assessing the stage of pregnancy in buffaloes[2]

Age in days	*Characteristic findings*
25-40 days	*Uterus in pelvic cavity; asymmetry of uterine horns, with pregnant horn 3-4 cm in diameter; membrane slip may be present and amniotic vesicle may be palpated.*
60 days	*Uterus at pelvic brim; pregnant horn 6-8 cm in diameter; membrane slip, amniotic vesicle present; cotyledons start to appear (1 cm).*
90 days	*Descent of uterus begins; pregnant horn 10-12 cm in diameter; hypertrophy of middle uterine artery (MUA) begins; cotyledons 1 x 2 cm.*
120 days	*Uterus mostly in abdominal cavity; foetus can be balloted; cotyledons 1.5 x 2 cm; MUA about 1 cm in diameter with amplitude mainly on pregnant side.*
150 days	*Uterus in abdominal cavity; foetus can be balloted orpalpated; MUA 1 cm diameter; cotyledons 2 x 2 cm.*
180 days	*Descent of uterus completed; foetus can be balloted in abdominal cavity: fremitus begins on the pregnant side; MUA 1-1.5 cm in diameter; cotyledons 2 x 3 cm.*
210 days	*Foetus palpable in the abdominal cavity; MUA on pregnant side 1-2 cm in diameter and fremitus present, while on the other side it is 0.5-1.0 in diameter with increased amplitude; cotyledons 3 x 4 cm.*
240 days	*Foetus palpable near pelvic brim; MUA 1.5-2.0 cm in diameter on pregnant side with fremitus present; cotyledons 3x5 cm.*
270 days	*Foetus palpable in the pelvic cavity or abdominal cavity; MUA 1.5-2 cm in diameter on pregnant side and 1 cm on the other side; fremitus present on both sides; cotyledons 5 x 3 cm.*

[2] Kuruwita *et al.* (1987)

300 days — *Large head of foetus palpable in pelvic cavity; suckling and pain reflexes present in the foetus; fremitus present on both sides, but may not be felt due to the pressure on vessels by the ascending foetus; MUA 1.5-2.0 cm in diameter; cotyledons 2 x 6 cm.*

12.3.6 Differential diagnosis of pregnancy

Errors of pregnancy diagnosis are definite possibilities. Failure to conduct the examination in a systematic manner is responsible most often for errors. The examiner should be able to differentiate pregnancy from the following conditions, which may provide signs suggestive of pregnancy; pyometra and hydrometra, white heifer disease, lymphoma of the uterus, foetal resorption, mummification and maceration and tumours of the ovary.

12.3.6.1 *Pyometra*: It is a form of chronic metritis characterised by the accumulation of purulent inflammatory exudate in the lumen of the uterus. The accumulation is most likely to be due to lack of relaxation of the cervix or to the presence of cervicitis combined with atony of the uterus resulting in the absence of expulsive forces. The amount of exudate could vary from as little as 25 to 30 ml which is barely felt upon examination, to several litres. Usually both horns are involved. The farmer may present a history of absence of oestrus and perhaps an intermittent leucorrhea. Usually there will be a history of abnormal parturition, either delayed or assisted delivery and/or retained placenta.

Visual examination of the perineum region will reveal the presence of signs of mucopurulent discharge from the vulva. The perineum region show evidence of discharge of mucopurulent material (e.g. scab formation around the perineum). Vaginal examination will reveal the oedematous and congested cervix with varying amounts of exudate in the anterior vagina. Per rectal examination will reveal distended uterine horns. The uterine horn is thinner than in the non-pregnant state, but thicker than in the pregnant state. Sometimes the ovary may carry a corpus luteum. This is because the postpartum ovary may resume follicular activity and hence go through ovulation before the completion of the uterine involution. However, if the uterus is inflamed, the pathological uterine endometrium may not be able to produce the luteolytic substance ($PGF_2\alpha$) in sufficient quantifies for the lysis of the CL subsequently (treatment will be discussed in a later section).

12.3.6.2 *Hydrometra*: This condition sometimes is presented with a history suggestive of a prolonged cystic ovarian condition (i.e. recurrent periods with signs suggestive of oestrus). This condition results from hypersecretion and accumulation of the uterine secretions and accompanied by extreme atrophy of the uterine wall. Uterine wall in extreme cases is almost paper thin and could be easily perforated during examination. The amount of fluid accumulated could range from a hardly detectable quantity to large quantities, sometimes up to two litres. The ovary may show one or more of large cystic follicles.

12.3.6.3 *White heifer disease*: This condition arises from the segmental aplasia of the genital tract which originates from the Mullerian duct. It is a developmental defect associated with a chromosomal abnormality. Originally, this was detected in the Short Horn breed where most of the heifers with white coats had this condition. Hence, the name of white heifer disease. However, it was later found that this condition could occur in other breeds too, not associated with white coats. The extent of aplasia and the number of missing segments are variable. Secretions from normal segments become entrapped between missing segments or anterior to the missing segment causing marked distension of the normal segments resulting in thin walls.

12.3.6.4 *Lymphoma of the uterus:* It is by far the most common tumour involving the uterus. The involvement of the uterus wall varies from a localised area in a single horn or in both horns. Occasionally, lymphatic proliferation attains a nodular form which may resemble cotyledons.

12.3.6.5 *Foetal resorption, mummification and maceration*: Prenatal or intrauterine death of the conceptus could occur at any time during pregnancy. Abortion, complete resorption, mummification and maceration are the means by which the body of the dam attempts to remove the dead tissue. Failure to deliver a calf at the expected time, based on the service date is the usual history given by the farmer.

(a) *Foetal resorption*: This condition is an extended process over a long period of time. Foetal resorption should be suspected when any one of the following signs are encountered; status of the uterus and its contents which do not correspond to the expected stage of pregnancy, reduced tonus of the uterine wall, diminished amount of foetal fluids and wrinkled and dehydrated foetal membranes.

(b) *Foetal mummification*: Foetal death is sometimes followed by persistence of the corpus luteum resulting in failure to expel the dead foetus. The foetal membranes become shrivelled and dry and the fluids of the allantois, amnion and foetus are resorbed. The uterus contracts on the foetus and moulds it into a dry contorted mass. This phenomenon is called mummification. The rectally detected features are a large uterus with no fluid, dry and wrinkled foetal membranes, and irregular and dry foetal mass, absence of cotyledons, small uterine artery without distinct pulses and most likely a CL in the ovary.

(c) *Foetal maceration*: Foetal death is occasionally followed by full or partial regression of the corpus luteum but the intended parturition may fail to occur. The partially open cervix would allow bacteria to gain entry into the uterus. The dead foetus then undergoes a gradual bacterial digestion and putrefaction. This phenomenon is called foetal maceration. This process continues until compact masses of foetal bones remain. The cervix remains patent and there may be a chronic mucopurulent vaginal discharge.

12.3.6.6 *Tumour of the ovary*: This is very rare in cattle and buffaloes. The most frequently encountered tumour is a granulosa cell tumour. Usually both ovaries are affected. The tumour may be as big as 25 to 30 cm in diameter. It is firm in consistency but small fluctuating areas indicating cystic areas may be present. Some areas of the tumour may secrete hormones which may cause changes in target tissues (e.g. development of mammary gland and initiation of lactation).

12.3.7 Steps in performing a rectal examination for diagnostic purposes

The examination should include the following steps in order to minimise errors.

Note: It is very important that the veterinarian performing the rectal examination must always wear protective clothing, disposable gloves and boots. The gloved hand must be lubricated well. The cows under examination must be restrained properly before attempting perform the examination.

12.3.7.1 *Careful examination of the breeding history*: This involves going through records, if available and careful questioning of the farmer with regard to service dates and history of the previous parturition.

12.3.7.2 *Visual examination of the animal*: Attention must be paid to the perineum area to detect any abnormalities such as discharges, swellings, etc., and also to the inguinal region to note the appearance of the udder. An assessment of the body condition (1 to 5 scale) must also be made at this stage (for information on body condition scoring system refer Chapter 28 on Body Condition Scoring System).

12.3.7.3 *Restraining of the animal*: A proper restraining method must be applied in order to minimise the movements of the animal. The perineum region must be thoroughly cleaned and the examiner must wear protective gloves (highly advisable) and apply a suitable lubricant prior to introduction of the hand into the rectum. This will minimise trauma and stress in the animal.

12.3.7.4 *Examination of the internal reproductive organs through rectal wall*: Within the rectum, remove all the dung by the back movement of the hand. Usually, the introduction of the hand into the rectum provokes a defaecation reflex. Ensure that the rectum does not get ballooned with air. When the rectum is relatively empty, locate the cervix and continue the examination of the organ systematically in the following order; cervix, uterine body, uterine horns and the ovaries. The normal characteristics of the different segments of the internal reproductive organs are given below in Table 12.5.

Table 12.5 Characteristics of different segments of the internal reproductive organs.

Structure/ component	Normal characteristics	Comments
Cervix	Muscular structure with abundant fibrous and elastic tissue. **Consistency:** hard **Shape:** cylindrical in shape, but may take different shapes such as cone-shaped or distorted contour. **Size:** length could range from 5 to 8 cm and diameter could range from 2-3 cm.	Any abnormality of the cervix could interfere with the passage of sperms/AI pipette.
Uterus	**Body:** Hollow muscular organ, continues as uterine horns. **Length:** usually 2-4 cm **Diameter:** usually 2-4 cm **Consistency:** Soft (or flaccid). But becomes turgid if it is under oestrogen influence. **Horns:** Hollow, muscular tube-like organ and tapering as uterine tubes. Usually symmetrical in size. But a slight asymmetry may be detected in pluriparous animals. **Length:** Each horn usually about 15-25 cm **Diameter:** Each horn usually about 2-4 cm **Consistency:** Soft (or flaccid) in consistency (in anoestrus as well as during luteal phase under progesterone influence), but become turgid if it is under estrogen influence.	Changes in shape, size and consistency of the uterine body and horns – suggest pregnancy or uterine pathology.
Ovaries	Tissue mass consisting of primordial and growing follicles, remnants of regressed follicles and corpora lutea (Corpora albicans), blood vessels, nervous and lymphatic tissue and covered by an epithelial tissue. **Consistency:** Usually hard. Contour and the surface could vary - smooth surface if no transient structures (CL or growing follicles) are present and lobulated or undulating surface if developing follicles or corpora lutea are present. **Shape:** Round/oval/elongated. Shape depends on the presence or absence of and types of transient structures. **Size:** Could vary from very small (inactive; e.g. 1-2x1-2x0.5-1 cm) to medium (functional if CL is present; 2-3 x 2-4 x 2-3 cm) to vary large (cystic; as big as a TT ball). **Transient structures:** Follicles and corpora lutea - refer notes in Section 12.3.3	The palpation of the ovary is the most important part of the examination. Further, recording of the findings in a systematic manner is very rewarding for repeated examinations as well as for the follow-up work.

The final diagnosis should be recorded with sufficient details giving at least a tentative diagnosis as shown in Box below.

Box 12.3 - Format for recording diagnosis

Following the completion of the rectal examination and careful and logical evaluation of the history, general clinical examination and rectal findings, the diagnosis must be given in the following manner.

e.g. Pregnant (and stage of pregnancy; refer Tables 12.3 & 12.4)
Non-pregnant; cycling (early-, mid- or late-luteal phase) or non-cycling.
Ovarian pathology (cystic/tumour)
Uterine pathology (endometritis -first-, second- or third-degree; metritis)
Development abnormaility (uterine/ovarian hypoplasia or segmental hypoplasia)

12.3.8 Other methods of pregnancy diagnosis

12.3.8.1 *Hormone measurement*: Measurement of progesterone in milk or blood sample collected on days 20 to 22 post-service, provide an indication of CL function. As shown in the diagram below (Fig.12.4), if conception has taken place, the CL formed from the ovulation would continue to remain functional and therefore the progesterone concentration would remain elevated (> 3 ng/ml). On the other hand, if

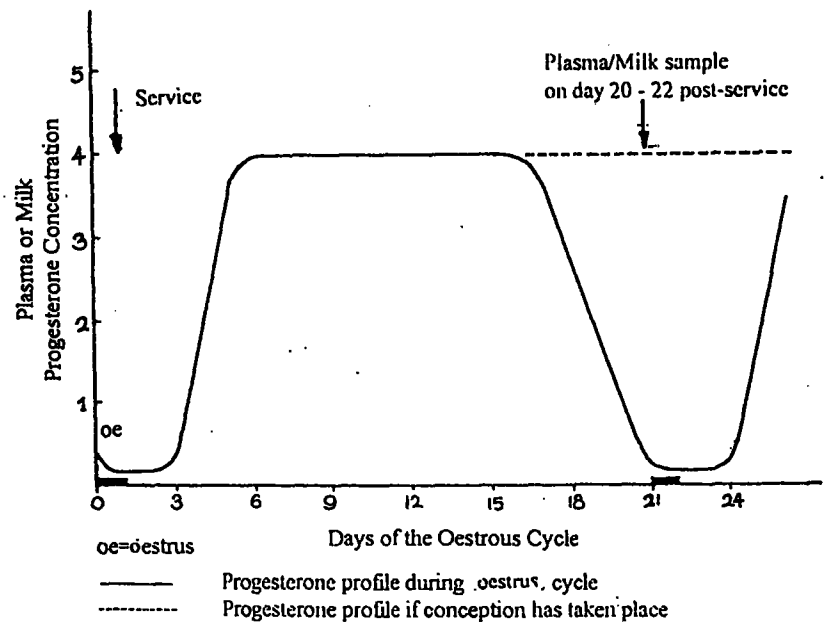

Fig. 12.4 Plasma /milk progesterone pattern during non-pregnancy and pregnancy.

the expected fertilisation has not taken place, then the CL from the previous ovulation undergoes lysis and the progesterone concentration will be at undetectable levels. However, this method may tend to give higher false positives as the variation in the length of the luteal phase may give moderately higher progesterone concentrations in day 21 post-service samples. But a low progesterone concentration in the sample is always indicative of non-pregnancy.

12.3.8.2 *Untrasound scanning*: This is the most accurate method of pregnancy diagnosis, but requires higher capital investment on the machine. It also requires skills to use the machine and interpret the findings. It is based on the principle that the ultrasound waves emitted by a probe would be reflected back by the tissues at different frequencies and intensities. The reflected waves are then received by a transducer which converts waves into a audible signal (e.g. Doppler method) or into a visual signal on a TV screen (real time scanning- A-mode or B-mode). This method could be used as early as day 25 of pregnancy.

Selected References

Arthur G.H., Noakes, D.E. and Pearson, H. (1982) *Veterinary Reproduction and Obstetrics* (5th edition). Baillierc and Tindall, London.

Bongso, A.R. (1977). *Lecture notes on Pregnancy Diagnosis.* Department of Veterinary Clinical Studies, University of Peradeniya.

Kuruwita, V.Y., Chandratilake, D., Mohan, V. and Perera, B.M.A.O. (1987) Criteria used for diagnosing the stage of pregnancy by rectal palpation in Lanka buffaloes. *Sri Lanka Veterinary Journal.* **35**, 39 [Abstract].

McDonald, L.E. and Pineda, M.H. (1989) *Veterinary Endocrinology and Reproduction.* (4th edition). Lea and Febiger, London, UK.

Peters, A.R. and. Ball, P.J.H. (1995) *Reproduction in Cattle* (2nd edition) Blackwell Science Ltd., Oxford, UK.

CHAPTER 13

Obstetrical Manipulations in Cattle and Buffaloes

H. Abeygunawardena

Introduction

Abnormal parturition (dystocia) may range from delayed parturition to a gross difficulty in delivering the calf. The outcome is usually either a dead calf, sick cow or loss of both the calf and the dam. Abnormal parturition could arise from a variety of causes: For descriptive purposes the causes of dystocia are grouped into two categories; maternal causes and foetal causes.

13.1 Causes of dystocia

13.1.1 Maternal causes

Breed/size incompatibility	- small dam/breed served by a large sire or sire of a large breed
Pelvic deformities	- congenital or acquired
Defective uterus	- toxic degeneration, strictures or scar tissues
Maternal disabilities	- general weakness of dam, due to diseases, weak abdominal muscles, torsion of uterus
Defective parturition mechanism	- incomplete dilatation of cervix
Prolapse of vagina	- acts as an obstruction to the passage of the foetus

13.1.2 Foetal causes

Breed/size incompatibility	- large foetus in a small pelvis (foeto-maternal incompatibility)
Foetal maldisposition	- abnormalities in presentation, posture and position
Foetal deformities	- defects in cranial and other body parts
Dead foetus	- dead foetus is unable to induce parturition mechanism

13.2 Steps in dealing with dystocia

A case of suspected dystocia must always be treated as an emergency, requiring a visit and examination as soon as possible. The attending veterinarian must be fully prepared to handle the case where the treatment may range from simple induction of parturition to caesarian operation or to exhaustive manipulation such as foetotomy.

13.2.1 Recording the history

A detailed history should always be obtained. This includes the age of the dam, parity, previous calving history, health status during pregnancy, especially during the

immediate pre-partum period, appetite, activity, date of service of present pregnancy, the expected date of calving, observed signs of impending calving and the time interval between the onset of signs and the present state, evidence of foetus and/or foetal membranes at the vulva, evidence of rupture of allanto-chorion or amnion with escape of fluids and the nature of any prior examination or attempt at delivery by farm personnel.

13.2.2 Clinical Examination

The cow should be adequately restrained in a suitable place, preferably in an open area with plenty of bedding. The following examination procedure is recommended.

13.2.2.1 *General clinical examination*: This should include a general assessment of the body condition and health of the cow, with particular emphasis on the evidence of hypocalcaemia or mastitis, assessment of status of vulva and pelvic ligaments for degree of relaxation, nature of any vulval discharge (particularly the smell) and examining the vulva for evidence for trauma from previous interference.

13.2.2.2 *Special clinical examination*: Internal examination of the vulva, cervix and the foetus must always be carried out with the attention of maintaining thorough aseptic conditions, as far as is possible. A suitable antiseptic solution and ideally a suitable lubricant must be used. Per-vaginal examination is usually rewarding, as it allows the examiner to determine whether the calf is alive and also the disposition of the calf (i.e. anterior or posterior presentation, flexion of limbs, neck and head, etc). A live calf always elicits a response to manipulation and also the heart beat and carotid pulses could be felt. This examination would also help to determine the integrity of the amnion and allanto-chorion. Further, it would be very beneficial to determine the degree of dilatation of the cervix.

13.2.3 Diagnosis

The history and clinical examination would allow the examiner to arrive at a diagnosis. The course and/or the underlying defect could be adequately established.

13.2.4 Treatment

It is not possible to provide a prototype treatment plan to suit all situations. There are several tools (i.e. ropes, torsion forks, foetotomes wires, etc.) and techniques (restraint, retropulsion, rotation, traction, caesarean operation, foetotomy, etc.) which could be used or applied depending on the type of dystocia and other conditions. The following guidelines would enable the attending veterinarian to handle the case with confidence.

13.3 Techniques and tools for handling a dystocia

There are very useful techniques and tools at the disposal of the veterinarian to handle dystocias in a more effective and professional manner.

13.3.1 Restraint

The movements of the animal should be adequately restricted by mechanical or chemical methods. If the treatment procedure is to be carried out on a standing animal, a stanchion with one adjustable railing must be used. If both sides are permanently fixed and the animal goes down, it could create problems to the veterinarian as well as to the animal. The animal must be tied with a head halter but not with a neck rope. In situations where chemical restraint is required, a high-dose (anterior) or low-dose (posterior) epidural analgesia could be used. Neither of these methods would interfere with uterine contractions but will abolish abdominal contractions to an some extent.

13.3.2 Intrauterine fluid replacement

This is perhaps the most important procedure that will prevent or minimise trauma to the birth canal resulting from friction during an assisted delivery. An assisted delivery must never be attempted when intrauterine liquids have been lost and the wall of the birth canal and the skin of the foetus are dry. Manipulation inside the birth canal becomes relatively easy when the foetus is in lubricant fluid. The simplest intrauterine liquid replacer is soap water. This could be prepared by dissolving a sufficient quantity of pure soap in warm water to the desired consistency (caustic soap or soap containing bleaching agents should be avoided). A suitable alternative preparation could be prepared by dissolving 10 gm of methyl cellulose in 5 litres of warm water. The artificial fluid must be introduced into the uterus using a flexible tube, preferably made of rubber. The outlet of the intrauterine liquid replacer is introduced into the genital tract as far anterior as possible over the dorsal aspect of the foetus with help of a flexible rubber tube and a pump. Alternatively, the fluid could be allowed to flow down into the uterus through gravity. The liquid should be replenished at frequent intervals. About 0.5 to 1.0 litre is usually sufficient at a time to permit manipulation and traction. It is important to remove as much of the introduced liquid replacer together with any uterine debris as is possible following completion of the manipulation. This may be achieved by either siphoning or stimulating uterine contractions using a stimulant like oxytocin.

13.3.3 Correction of faulty disposition

The normal disposition of the calf in a natural delivery is an *anterior presentation in dorsal position with extended fore limbs and neck with hind limbs flexed under the body*. A posterior presentation with extended hind limbs could occur without creating serious problems. As in many instances, dystocia is due to abnormalities in foetal disposition. The foetus should be placed in normal disposition before attempting to force the deliver of the calf. Corrections should be attempted per vagina. This could either be done by hand alone or with the use of specialised obstetrical instruments. As a jammed foetus would be tightly placed against cervix and pelvic region, the foetus must be pushed back into the birth canal *(retropulsion)* in order to create sufficient space for manipulation (Fig. 13.1).

Correcting faulty dispositions is easy when the calf is alive, as it would show spontaneous movements during manipulation. The steps and procedures for correcting

a faulty disposition before a forced delivery is attempted vary from case to case and would most likely depend on the type of abnormality. It is difficult to give a rigid stereotype treatment protocol but the guidelines given here (refer the Table 13.1) would be helpful in many instances.

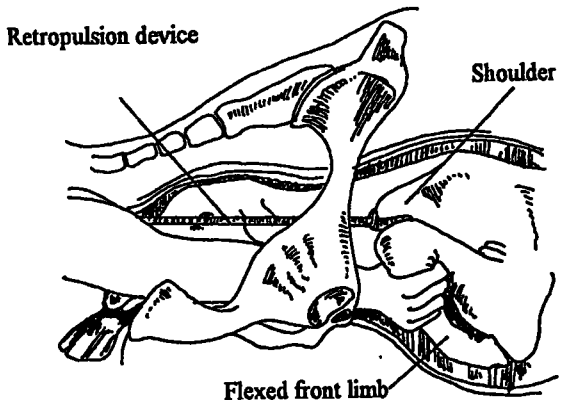

Fig. 13.1 Technique of retropulsion where the foetus is repelled using a crush while the faulty disposition is corrected by hand.

13.3.4 Traction

The term traction implies the application of force to remove the calf from the mother's birth canal, either by hand or using ropes or chain snares. The traction force is usually applied to the head with head snares or to limbs with limb snares (Fig. 13.2). The limb snares are usually placed around the pasterns and the head snares behind the ears and the occiput. The traction force must stimulate and synchronise with the dams expulsive forces. Further, the initial direction of the pull must be upward and caudal until the foetal head is engaged into the pelvic cavity and thereafter in a caudal and ventral direction (Fig. 13.3). The limbs must be placed in such a way that one leg is

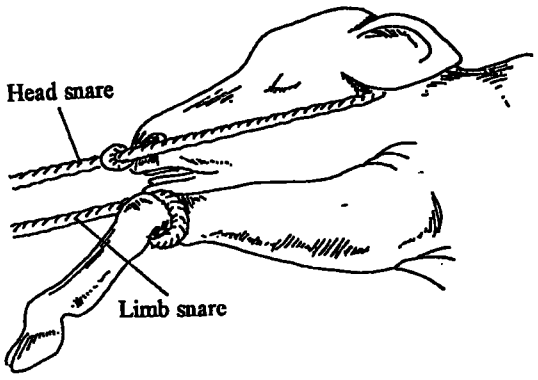

Fig. 13.2 Sites of attachment of ropes snares for traction: (a) head snare for application of traction to the head and (b) limb snare for application of traction to the limb.

slightly advanced in relation to the other and force is applied alternatively to the limbs (Fig. 13.4).

If delivery per vaginum is not possible, then other methods need to be considered. The type of intervention or technique depends on whether the foetus is alive or dead. If the foetus is alive then the choice is a caesarian operation, but if the foetus is dead then the choice is foetotomy, provided sufficiently skilled staff and the necessary instruments are available.

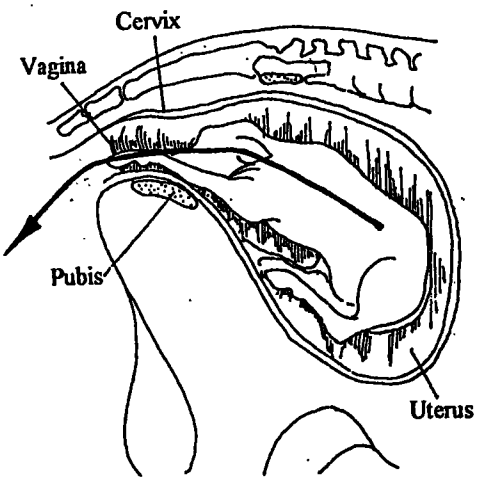

Fig. 13.3 Diagram showing direction of the traction force which must follow an arc: (a) initial traction must be directed upward and caudal direction until the head engages into the pelvic cavity and then (b) direction of traction must be in the ventral and caudal direction.

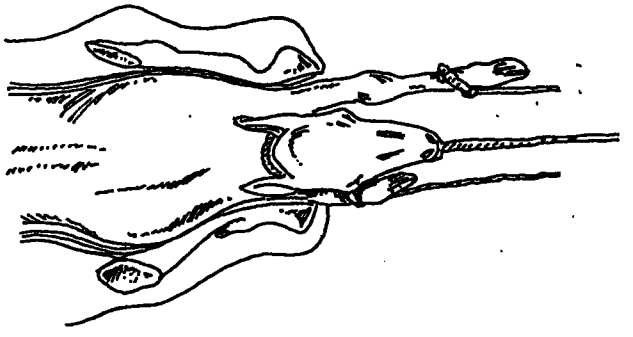

Fig.13.4 Diagram showing the position of limb and head snares and positions of two limbs where traction are applied in alternate fashion.

13.3.5 Caesarean operation (see section 13.5).

This implies a delivery by surgical means, through an opening in the body wall and uterus.

13.3.6 Foetotomy (see section 13.6).

This implies the severing of the foetus into small pieces and removing the foetus part by part. Severing of the foetus is done with specialised instruments.

13.4 Guidelines for correction

13.4.1 Foeto-maternal disproportion

This is the commonest cause of dystocia, particularly following cross-breeding of indigenous animals with bulls of large *Bos taurus* breeds. The foetus is large compared to mother's birth canal. The foetus is presented in normal disposition (anterior or posterior presentation in dorsal position with extended head and limbs). Usually there will be a history of unproductive straining and perhaps foetal parts (fore limb and or muzzle or hind limbs) may be visible at the birth canal. Upon examination, the veterinarian will find that the vulva, vagina and cervix are adequately relaxed and the uterine contractions are present with sufficient vigour. However, this would depend on the time lag between the onset of parturition and examination, as in protracted cases, uterine inertia could set in due to exhaustion. The foetus may be alive or dead. The life of the foetus could be in jeopardy, within a couple of hours of the onset of parturition, as the supply of oxygen and metabolic substrates become inadequate and as placental separation commences and proceeds.

13.4.1.1 *Treatment:* Adequate restraint of the animal is necessary, as a precautionary measure. Epidural analgesia should not be employed, as this would abolish abdominal straining, which may be very useful in complementing the force of manual or mechanical extraction of the foetus.

(a) If the water bags have ruptured, a intra-uterine fluid replacer must be introduced.

(b) Apply traction, if the foetus is in anterior presentation and apply ropes or chain snares to the pastern with their eyelets on the dorsal surface of the fore feet. Apply traction, initially in an upward and then subsequently in a downward direction as the foetus enters the birth canal. If the foetus is jammed at the pelvis (hip lock sometimes could occur with large foetuses as the greater trocanter of the femur impinges on the shaft of the ilium and the stifle impinges on the pelvic brim). In this situation, repel the foetus backward into the uterus and rotate along its longitudinal axis through 45 to 90 degrees. Apply traction again.

If the foetus is in posterior presentation, apply the ropes or chain snares to pastern with the eyelets on the ventral surface of the foetal

hind feet. Rotate the foetus through 90 degrees (clock or anticlockwise) to a dorso-lateral position. This is done to align the greatest diameter of the foetal pelvis with the greatest diameter of the maternal pelvis. Then apply traction in the same way described for anterior presentation. Once the foetus enters the birth canal, it may be desirable to rotate the foetus to the original dorso-dorsal position.

(c) Failure to deliver a live calf in this manner may compel the veterinarian to resort to a caesarian operation. If the foetus is dead, he/she may choose to do foetotomy, provided the necessary equipment is available.

13.4.1.2 *Prevention*: Foetal oversize in relation to the maternal pelvis has become a common problem among the Bos taurine cattle population with pregnancies resulting from AI or natural service from larger bulls. Hence advice on dam and sire selection must be provided to the client. The problem of foetal oversize could be minimised to an extent by avoiding overfeeding the late pregnant cow. This not only reduces foetal size but also reduces fat deposition around the pelvis. In situations where foetal oversize is a problem, farmers may seek the help of a veterinarian to induce parturition.

13.4.2 Partial or incomplete dilatation of the cervix

The dilatation of the cervix may become compromised in some situations. There are several underlying or precipitating causes such as hypocalcaemia, fibrosis of the cervix following injury and endocrine and neurological dysfunction. There will be a history of unproductive straining. Clinical examination will reveal a partially dilated cervix with parts of the calf protruding through the partially open cervix.

13.4.2.1 *Treatment:* If the animal is in the protracted first stage of parturition and the foetus is alive, administration of calcium borogluconate may be attempted as the first step. In the absence of acute signs of hypocalcaemia, administration of calcium borogluconate may be attempted through the sub-cutaneous route, to avoid any adverse affects on cardiovascular function. If the animal fails to respond to the treatment, manual dilatation of the cervix could be attempted. If the cervix responds to manipulation, then the calf could be extracted by applying manual force. Failure to deliver the calf by the above means may compel the veterinarian to resort to a caesarean operation.

13.4.3 Uterine torsion

Uterine torsion is a complication of the second stage of parturition. The heavy uterus may not be able to hold its position with its ligaments, particularly if there is heavy foetal movements. The foetus under these circumstances tends to rotate either in a clock or anticlockwise direction. Usually left rotation is more common. There may be a history of unproductive straining and the cow tends to remain standing and show signs of great discomfort.

13.4.3.1 *Clinical Findings*: Clinical examination of the perineum and vulva will show a slight distortion and pulling into the pelvis. Vaginal exploration by hand may be difficult as the torsion may obliterate the vaginal vault. The directional twist of the vagina and cervix will indicate the direction of rotation.

13.4.3.2 *Treatment*: This could be attempted through either of two methods.

(a) *Rotation of foetus through vagina*: This is attempted if the cervix is open and the foetus is alive. Insert the hand through the constriction of the anterior vagina and partially dilated cervix. Then grasp the foetus at the shoulder or elbow and rotate the foetus in the opposite direction to the twist. Alternatively, rotation of the foetus could be attempted by means of a torsion fork .

(b) *Rotation of the cow*: Correction of the twist by rotating the whole cow has also been attempted with successful results. The aim of this procedure is to rotate the cow's body as quickly as possible in the direction of the torsion while the uterus remains relatively steady. The cow is cast on the side towards which the torsion is directed (i.e. left rotation - left lateral recumbency). Tie the front and hind limbs separately. While the head is held tightly, the cow is pulled in the direction opposite to the rotation by pulling the ropes fixed to fore and hind limbs simultaneously. If the foetus is accessible, then hold the foetus in its existing position, while the cow is being rotated. Following the complete rotation of cow's body by 180 degrees, ascertain the progress through the vagina. If the correction has been effected, the examiner will be able to reach the foetus without any hindrance. If the rotation has not been effected, the cow must be returned to its original position and the procedure must be repeated. Once the torsion is corrected, the calf could be delivered with manual assistance. If rolling fails to correct the abnormality, delivery by caesarean operation must be attempted.

13.4.4 Uterine inertia

Uterine inertia arises if the uterus of the animal fails to generate innate and sustained rhythmic contractions to force the foetus out through the maternal birth canal. This could occur as a result of an innate deficiency in the uterine musculature (primary uterine inertia) which is common in old cows or also in cows with hypocalcaemia. It could also occur as a consequence of a protracted first stage of labour (secondary uterine inertia). In such circumstances, a clinical examination will reveal that the calf may be in normal disposition, with fully opened cervix, but no muscular contractions.

13.4.4.1 *Treatment*: Administration of calcium borogluconate is recommended, if hypocalcaemia is suspected. If there is no response to intravenous administration of calcium borogluconate, then delivery by traction must be attempted.

13.4.5 Dystocia due to abnormal foetal disposition

Dystocia due to abnormal disposition (presentational, positional and postural defects) are very common among dairy cattle. Suspected cases, usually have a history of unproductive straining and perhaps some evidence that the foetal extremities, head or limbs are positioned at the vulva. Vaginal examination will reveal that the cervix is fully dilated and uterine examination will reveal that the calf delivery process is obstructed because of a defect of its disposition. Careful examination *per vaginum* will enable the examiner to determine the exact abnormality. The cause(s) of abnormal disposition is not fully understood. Perhaps this is due to failure of development of reflexes, inadequate stimulation of foetal movements or foetal anoxia. These abnormalities could be either unilateral or bilateral, involving the head, neck or limbs or a combination. The suggestive measures are given in the Table 13.1 below.

13.5 Caesarean operation

Caesarean operation is a surgical procedure whereby the foetus is removed through an abdominal and uterine incision.

13.5.1 General Indications

(a) When a vaginal delivery is not possible or cannot be achieved without either loss of the foetus or causing irreparable damage to the maternal birth canal, a caesarean operation may be the choice. Indications include dystocia due to foeto-maternal disproportion, incomplete dilatation of the cervix or irreducible faulty disposition of the foetus.

(b) It is also undertaken electively to relieve the dam of the foetal load when a severe pre-parturient disorder exists - for example mummified foetuses, dropsy of the foetal sacs, pre-partum recumbancy associated with ketosis, hypocalcaemia and downer-cow syndrome and prolapse of vagina and cervix.

13.5.1.1 *Complications*: The incidence of death following post-surgical complications increases with the time interval between the onset of second stage labour and the operation, from 2 to 15 percent within 18 hours, to 18-40 percent after 18 hours. According to many authors 50 to 75% of cattle undergoing caesarean operation can be bred again. Abortions during subsequent pregnancies have been observed up to 20% of the cases. Further, a higher than usual occurrence of incomplete dilatation of the cervix or weak uterine contractions have been observed during subsequent parturitions. This is presumably due to the disruption of the normal myometrial fibre pattern in the vicinity of the uterine incision and to peri-uterine adhesions. High incidence of adhesions between the peritoneum and the uterus have been reported post-operatively and this does appear to influence future reproductive function.

13.5.1.2 *Location of the operation*: All efforts must be made to perform the operation in a clean and well lighted and ventilated place. Adequate shelter, restraint facilities

and reasonable hygienic conditions must be provided. Occasionally, the operation may be performed on pasture when the dam is recumbent and cannot be moved. However, surgical risk and post-operative complications are greatly increased when the patient is recumbent in an inconvenient place and in uncongenial situations.

13.5.1.3 *Instruments*: The minimal requirement of equipment/instruments that must be available are as follows:.

Surgical towels and towel clips
Scalpel handle and scalpel blades
Haemostat (artery forceps) 6-8 numbers
Needle holder
Uterine and tissue forceps
Blunt pointed scissors
Obstetrical chains or rope snares
Chromic catgut (number 3 or 4)
Extra-heavy non-absorbable suture material (silk or nylon- number 4)
Suture needles
Hypodermic needles 16-18 G, 1 1/2"
Lignocaine 2% solution
Suitable antibiotic preparation
Suitable antiseptic solution
Shaving razor and a blade or suitable large animal clipper

13.5.1.4 *Operational procedure*: Three main approaches may be used for caesarean operations in the cow. (i) flank approach; (ii) ventro-lateral approach and (iii) ventral midline approach.

The advantages and disadvantages of the each method are given in the Table 13.2 below.

13.5.2 Left flank operation

This could be performed on a standing cow for dystocia with a live or dead foetus but not with a decomposed foetus.

13.5.2.1 *Restraining procedure*: Place the animal with its right side against a wall, fence or rail and the head secured with a head halter. A large rope may be applied to the right hind limb, passed between the forelegs and tied to the right side of the halter, so that if the cow goes down, she will become recumbent on the right side. It may be desirable to induce epidural analgesia to immobilize the tail region and also to prevent defaecation.

13.5.2.2 *Preparation of surgical site*: Operation area is a rectangular area extending ventrally from a line between the lateral point of the first lumbar transverse process and the tubar coxae to about 10 cm below the level of the patella. This area should be clipped or shaved and thoroughly scrubbed and disinfected with an antiseptic solution (5% tincture of iodine).

Table 13.1 Suggestive measures for handling dystocias due to foetal maldispositions

Abnormality	Suggestive measures
(A) Anterior presentation	
1. Unilateral or bilateral carpal flexion	(a) Employ adequate restraint and epidural analgesia as described in section 14.1 (b) Repel the foetus into the uterus by pressing at the shoulder or head. Retropulsion must be maintained during correction. This could be done either by hand or by using a crutch. (c) Grasp the retained foot and push the carpus upward. (d) The foot is then carried outwards and finally brought forward in an arc over the pelvic brim. Extend the foot alongside the other limb. In order to prevent damage to the genital tract while the limb is brought forward, the foot must be held with the cupped hand. In the case of bilateral carpel flexion, repeat the steps (b) through (d). (e) Deliver the calf by traction using ropes or chains as described in section 14.2.
2. Unilateral or bilateral elbow flexion	(a) Same as 1.a (b) Repel the foetus as described in 1.b. (c) Grasp the retained foot and pull it over obliquely in an upward direction arching over so as to lift the olecranon process over the maternal pelvic brim. Place the limb alongside of the other limb. In the case of bilateral elbow flexion, repeat the steps (b) and (c). (d) Deliver the foetus by traction
3. Unilateral or bilateral shoulder flexion	(a) Same as 1.a (b) Repel the foetus as described in 1.b (c) Grasp the retained fore leg and convert the shoulder flexion into a carpel flexion. Then correct the carpel flexion as described in 2.c. In the case of a bilateral shoulder flexion, repeat the steps (b) and (c). (d) Deliver the foetus by traction
4. Lateral flexion of head and neck	(a) Same as 1.a (b) Repel the foetus as described in 1.b (c) Grasp the muzzle of the calf and bring it over the pelvic brim through an arc until the nose is in line with the birth canal (d) Deliver the foetus by traction
5. Ventral deviation of head	(a) Employ restraint and epidural analgesia as described in 1.a (b) Repel the foetus by pressing at the fore-head (c) Grasp the mandible of the foetus and bring it over the pelvic brim (d) Deliver the foetus by traction
(B) Posterior Presentation	
6. Unilateral or bilateral hock flexion	(a) Apply restraint and epidural analgesia as described in 1.a (b) Repel the foetus by pushing at the perineum

	(c) Grasp the foetal foot and bring it backward through an arc over the pelvic brim. The digit of the foot must be cupped by hand to prevent possible irritation or damage to the uterus or alternatively, apply a rope or chain snare to the pastern and place the eyelet on the dorsal surface of the foot and pass the end piece of the snare between the digits and apply traction to the snares while the foetus is being repelled back.
	(d) Deliver the foetus by traction
7. Unilateral or bilateral hip flexion	(a) Apply restraint and epidural analgesia as described 1.a.
	(b) Repel the foetus by pushing at the perineum
	(c) While retropulsion is maintained, grasp the foetal leg near to the hock. Convert the hip flexion to one of hock flexion. Correct the hock flexion as described in 6 c .
	(d) Deliver the foetus by traction
(C) Postural abnormalities	
8.Anterior presentation in lateral position or anterior presentation in ventral position	(a) Apply restraint and epidural analgesia as described in 1.a.
	(b) By means of the thumb and middle finger - press the eye ball which will cause a convulsive reflex response. Then apply the rotational force in the appropriate direction, holding by the head and turning the foetus into dorsal position.
	(c) Deliver the foetus by traction
9. Postperior presentation in lateral or vental position	(a) Apply restraint and epidural analgesia as described in 1.a.
	(b) Grasp the stiffle region of the hind limb and by simultaneous retropulsion and rotation convert foetus into a dorsal position
	(c) Deliver the foetus by traction
(D) Abnormal disposition due to presentational abnormalities	
10. Oblique dorso-ventral or oblique ventro-vental presentation	(a) Apply restraint and epidural analgesia as described in 1.a.
	(b) Convert the abnormality into anterior or posterior longitudinal presentation. Following that an attempt must be made to bring the foetal extremities into the pelvic cavity. First convert the defect into ventral longitudinal presentation. The foetus then can be rotated to a dorsal position as described earlier.
	(c) Deliver the foetus by traction
11. Dorso-transverse or ventro-transverse presentation	(a) Apply restraint and epidural analgesia as described in 1.a.
	(b) Repel the foetus and try to advance the nearest extremity to the birth canal. First, an attempt must be made to bring the foetus to a ventral anterior or ventral posterior presentation. The next step is to convert this into a dorsal position. In the case of a ventro-transverse presentation, convert the abnormality into longitudinal, usually posterior presentation-ventral position. This involves repelling the anterior extremity while the posterior extremity is advanced. Then convert the ventral position into a dorsal position.
	(c) Deliver the foetus by traction

Table 13.2 Advantages and disadvantages of different approaches for a Caesarean operation.

Feature	Flank	Ventro-lateral	Ventral midline
(a) Analgesia and anaesthesia	Epidural and local infiltration	Epidural, local infiltration plus some degree of narcosis and sedation are required.	Epidural, local infiltration plus some degree of narcosis and sedation are required.
(b) Accessibility of the uterus	Difficult to exteriorize	Easy to exteriorize	Easy to exteriorize
(c) Possibility of extension of incision	Unlimited	Limited by ventral abdominal vein	Unlimited
(d) Danger of postoperative herniation	Minimal	Considerable	Considerable
(e) Contamination of peritoneal cavity with uterine liquids	Unavoidable	Easily avoidable	Easily avoidable

13.5.2.3 *Induction of regional or local infilteration analgesia*: A suitable anagesic method is employed by performing either a proximal or distal paravertebral nerve block or an inverted 'L' block. With a very nervous or aggressive animal, a light dose of xylazine may be given to induce mild sedation.

13.5.2.4 *Surgical procedure*:

(i) Operational area is draped with a large sterile animal laparotomy sheet (you may not have this luxury, but any improvised material is suitable)

(ii) A vertical incision of 25 cm long is made in the left flank commencing at about 8 to 10 cm ventral to the edge of the lumbar transverse process and 5 cm caudal to the most posterior point of the last rib. Control haemorrhage with haemostats or by ligating vessels.

(iii) A small incision is made with a scalpel on the exposed oblique abdominal muscle and deepen to go through the both muscles and then enlarge the opening using blunt scissors.

(iv) The exposed transverse muscle is lifted along with the peritoneum with a help of a tissue forceps and nicked through with a scalpel. The incision is then enlarged with scissors.

(v) Approach the uterus through the opening. It may be necessary to displace the posterior portion of the rumen anteriorly and medially and the dorsal edge of the large omentum ventrally. With the hand, the alignment of the foetus must be assessed. In most cases, the uterus can be exteriorized by grasping the most anteriorly positioned (in relation to the laporatomy opening) foetal limb together with the overlying section of the uterine wall and pulling through the incision. Thus, a hind limb is usually exteriorized in the case of anteriorly presented foetus and the fore limb is exteriorised in the case of posterior presentation. When the uterus cannot be exteriorized in this way, an attempt must be made to pass one hand under the uterus and lift it towards the incision while maintaining a hold on the foetal limb with the other.

(vi) As soon as the uterus is sufficiently exteriorized, it is secured by a pair of uterine forceps applied above the level of foetal foot. A small incision is then made with a scalpel through the uterine wall overlying the foetal inter-digital cleft. The incision is then enlarged with scissors to at least 25 cm, while care being taken not to cut placentomes.

(vii) Obstetrical snares are then applied to the limb and the foetus is delivered by traction. No attempt should be made to remove the foetal membranes. However, loose parts may be removed.

(viii) While the uterus is fixed with two pairs of uterine forceps (one at each end of incision) which are held by an assistant, the incision is closed with a double row of continuous inverting sutures. Before closing the uterus and 1 to 2 uterine pessaries containing a suitable antibiotic or a combination is inserted into the uterus.

(ix) Uterus is then cleaned and washed with normal sterile saline and replaced in the abdominal cavity. To minimize adhesion formation, intra-peritoneal administration of corticosteriods is recommended.

(x) Close the abdominal cavity in the following manner. First the peritoneum and transverse abdominal muscles are sutured as one layer using continuous sutures of *chromic catgut*, followed by suturing both oblique muscles together using continuous sutures with chromic catgut. Then place a row of subcutaneous sutures, again using chromic catgut. Finally, the skin is closed with interrupted vertical mattress sutures or with continuous blanket sutures using heavy synthetic suture material.

13.5.2.5 *Post operative care*: (i) Administer parental antibiotics - broad spectrum single or combination of antibiotic for 5 to 7 days; (ii) Examine the animal at frequent intervals (every other day) for post operative complications - such as infection of surgical site and placental retention and treat accordingly.

13.5.3 Ventro-lateral caesarean operation with the cow in lateral recumbency

This procedure is used when the foetus is dead and decomposed or mummified.

13.5.3.1 *Restraining procedure*: The cow has to be cast either with ropes following sedation (with a suitable sedative like xylazine hydrochloride) or by general analgesia (e.g. IV chloral hydrate). Use of xylazine, however produces undesirable side effects, elevating myometrial tone so that manipulation of the uterus become difficult or sometimes even impossible. The recumbent and sedated or unconscious cow is then repositioned on her right side with the left hind leg tied in abducted and backward extended posture.

13.5.3.2 *Analgesic method*: Local analgesic solution is infiltrated parallel and dorsal to the proposed incision line.

13.5.3.3 *Surgical procedure*:

(a) The laparotomy incision is made on a line drawn from the patella to umbilicus and run about 8 cm lateral and parallel to the left milk vein and the dorsal border of the mammary gland. This approach permits access to the uterus at any stage of pregnancy.

(b) Through the skin incision, a small incision is made with a scalpel through the muscle, rectus abdominus and the peritoneum. The incision is then enlarged with blunt pointed surgical scissors. The uterus is covered with omentum and lies directly under the parietal peritoneum.

(iii) After displacing the omentum anteriorly the uterus is exteriorized and incised over the foetal limb. The foetus is then delivered as described for the left flank operation.

(iv) The rest of procedure is same as described for the left flank approach.

13.5.4 Ventral midline caesarean operation

This procedure is useful to remove dead and decomposing or mummified foetus.

13.5.4.1 *Restraining procedure:* The procedure is same as for the ventro-medial operation except that the animal is positioned on her back with both hind legs extended and secured posteriorly.

13.5.4.2 *Analgesic method*: Procedure in the same as described for ventro-medial operation.

13.5.4.3 *Surgical Procedure*: A midline incision is made which starts immediately in front of the udder and extended for about 35 cm anteriorly. The incision is then extended as far as the xyphoid cartilage when a large opening is needed for delivery of an extremely emphysematous foetus. The abdominal cavity is opened by making a small incision with a scalpel through the linear alba. The incision is enlarged with blunt-pointed surgical scissors. After displacing the omentum the cow is tilted through 45 to 90 degrees to the left side so that the uterus may be partly exteriorized into a sterile surgical sheet or drape. Delivery of the foetus and the closing of the uterus are accomplished as in the previous methods. The peritoneum and facia are then closed with simple interrupted sutures, placed at about 1 cm apart, using thick chromic catgut. This is necessary because of the risk of prolapse of internal organs following wound breakdown. The skin incision is then closed by interrupted vertical mattress sutures.

13.5.4.4 *Post-operative care*: Same as described for flank approach.

13.6 Foetotomy

Foetotomy is the reduction of the foetus by the removal or destruction of foetal parts in order to permit the delivery. This procedure is strictly performed on a dead foetus and therefore is indicated to preserve the life of the dam for future breeding or fattening or slaughter. This procedure nevertheless has certain advantages and disadvantages. Foetotomy creates certain dangers to the dam; i.e. injury to the birth canal from instruments or projections of severed foetal bones. Often it is a prolonged, exhausting and tedious procedure. To the operator, foetotomy often means a protracted and disagreeable procedure, involving the risk of injury to himself by sharp, severed bones and infection from a decomposing foetus. Nevertheless, foetotomy is one of the most valuable obstetrical operations. However, it requires skill, patience and endurance. Reports indicate that fertility of cows following foetotomy may be depressed by 25%, but it may be higher if the foetus is in a decomposing state.

Foetotomy can be performed in two ways.

13.6.1 Subcutaneous or intra-foetal foetotomy

It involves the use of the foetotomy knife and removal of skeletal, muscular and visceral structures to reduce the size of the foetus in order to deliver the foetus through the vagina. Intra-foetal foetotomy is difficult to perform and is usually considered with severely oedematous foetuses where intrauterine manipulation is severely restricted. Caesarean operation is also contraindicated in this condition, due to danger of the contamination of the peritoneal cavity with exudate from the decomposing foetus.

13.6.2 Percutaneous or extra-foetal foetotomy

This involves the sectioning of the foetus by cutting through the skin, muscle and bones with a foetotome wire. This method has advantages over the subcutaneous method as it is faster and easier to perform. Limitations of this method are the greater risk of injury to the birth canal by use of instruments and from sharp bones which may protrude after a portion has been removed.

13.6.2.1 *Indications*: (a) Foetomaternal disproportion, (b) Irreducible maldisposition (presentational, postural and positional defects) and (c) Foetal malformation

13.6.2.2 *Instruments:* (a) Palm knife, (b) Wire-saw foetotome, (c) Foetotome wire and handles, (d) Obstetrical chains or snares, (e) Eye hooks, (f) Intrauterine liquid replacer, (g) Hand gloves and (h) Antiseptics and antibiotics.

13.6.2.3 *Method*:

(a) Animal must be restrained in a suitable place (stanchion, against a railing, wall etc). To reduce straining caudal epidural analgesia is employed.

(b) Intrauterine lubrication must be done with a suitable fluid to facilitate the manipulation of the foetus and minimize trauma to the birth canal.

(c) The procedure from here onwards vary with the type of dystocia. However, the principles of using the instruments and the procedure of severing the foetal parts are the same.

To cut extended parts (i.e. limb, head),

(i) Pass the foetotome wire through both foetotome tubes forming a loop,
(ii) Slip the loop of foetotome wire threaded through the foetotome over the foetal part and hold it over the site of the intended cut and reduce the diameter of the loop working with the free ends of the wire.
(iii) Place a chain or rope snare over the pastern in the case of the limbs or over the lower or upper jaw in the case of the head.
(iv) Cut the foetal part with the wire by applying sewing movement with the handles attached to the free ends of the wire, while holding the wire at the right place, by holding the proximal end of the foetotome with the operator's fingers.

To severe a joint or split the foetal trunk into pieces,

(i) Foetotome wire is passed through one tube of the foetotome and attached the free end to a wire introducer,.

(ii) Pass the wire introducer over the foetal part (i.e. over the shoulder in the case of the anterior presentation or over the rump in the case of posterior presentation) and recover it from the underneath the foetal body while ensuring that a loop is formed over the site of the intended cut (i.e. over the body trunk, over the shoulder). The introducer is retrieved and brought backward and then the free end of the wire is threaded through the other tube of the foetotome.

(iii) Hold the foetotome tightly against the site of the intended cut and reduce the size of the loop of the wire by working with the free ends of the wire. Then apply cutting force to severe the part.

(iv) Extract the severed part by fixing a snare through a hook.

(iv) The process is repeated until the foetus is reduced to an appropriate size for removal through the vulva. The number of cuts required vary and depend on the type of dystocia and whether the foetus is emphysematous or not.

The following are suggested cuts in dealing with different types of dystocia.

Anterior presentation:

Amputation of fore limbs
Amputation of hind leg
Amputation of head
Transverse bisection through the mid thorax
Longitudinal bisection of the foetal pelvis

Posterior presentation

Amputation of hind leg
Transverse section in the lumbar and thoracic areas
Oblique bisection of the anterior part of the foetal body

Selected references

Arthur, G.H., Noakes, D.E. and Pearson, H. (1982) *Veterinary Reproduction and Obstetrics* (4th edition). Bailliere and Tindall, London.

Jackson, P.G.G. (1995) *Handbook of Veterinary Obstetrics* W. B. Saunders Company Limited, London, UK.

Noakes, D. E. (1986) *Fertility and Obstetrics in Cattle.* Blackwell Scientific Publications, London. UK.

Roberts, S.J. (1971) *Veterinary Obstetrics and Genital Diseases* (2nd edition). Published by author, New York.

CHAPTER 14

Reproductive Disorders in Cattle and Buffaloes

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Introduction

Reproductive functions could be derailed at various stages. Disorders could occur during the prepubertal period, pregnancy, parturition and postpartum period. These disorders result in losses through delayed puberty, conception failures and embryonic and foetal deaths. The most frequent and important disorders and defects are discussed in this chapter in chronological order from the embryonic stage to the postpartum period.

14.1 Embryonic death

During the period from conception to day 43, the conceptus is referred to as the embryo and beyond that, it is referred to as the foetus. Death which may occur before day 13 is referred to as an early embryonic death (EED) while death between day 14 to day 43 is referred to as a late embryonic death (LED).

14.1.1 Early embryonic death

The embryo arrives at the uterus between 72 to 96 hours after fertilisation and the conceptus by this time is at the 8 to 16 cell stage. The conceptus is surrounded by the zona pelucida, and floats freely within the uterus and between days 9-11. It hatches out from the zona pellucida and elongation commences. While it is still in the free floating stage, by about day 21 or so, the process of apposition and adhesion prior to implantation commences.

Early embryonic death could be the result of variety of reasons:

(a) Ageing of gametes
(b) Genetic incompatibility
(c) Stress - hot, humid weather and gross starvation
(d) Pyrexia as a result of generalised infection - viral, bacterial or haemoptrophic parasitic diseases
(e) Fatty liver syndrome - A condition which is characterised by excessive mobilisation of depot fat and deposition in liver parenchyma. This condition has been observed in cows that are overfed during the prepartum period but underfed after calving, presumably resulting from excessive utilization of body fat to meet nutrient requirements.
(f) Nutritional imbalances - Nutritional excesses or deficiencies probably influence embryonic survival by altering the uterine environment
(g) Uterine infections - There are a variety of non-specific and specific infections which could affect embryonic survival by directly or indirectly altering the uterine environment. Non-specific organisms

like corynebacterium species, streptococci and staphylococci and *E. coli* could infect the uterus and uterine tubes creating an adverse environment for the embryo. In addition, specific organisms which have a predilection for the pregnant uterus, such as *Trichomonas foetus* (direct and indirect effects), *Camphylobactor foetus* (direct effect), *Infectious bovine rhinotracheitis/infectious pustular vulvo vaginitis (IBR/IPV)* virus (indirect effect).

(h) Endocrine deficiencies - Progesterone deficiency conditions may arise due to sub-normal luteal function. This may be very frequent in oestrus following hormonal induction and synchronisation.

Following the death of the conceptus, the conceptus and the associated membranes may undergo autolysis and are usually resorbed completely. The cow will return to oestrus at the normal interval (i.e. in 21 days) and the condition is referred to as "repeat breeder". This condition, therefore is impossible to differentiate from fertilisation failure which may result from other factors (e.g. wrong timing or technique of insemination, poor quality of semen, etc.).

14.1.2 Late embryonic death

Death occurs between day 14 and 43. The possible causes are the same as those listed under early embryonic death. The difference in this instance, is that the animal will return to oestrus not by about day 21 but at a later date, resulting in prolonged inter-service intervals. If a large number of animals are involved and there is evidence of mucopurulent discharge, then the possibility of specific infections such as camphylobactor or trichomonas infection could be suspected.

14.2 Foetal death

Foetal death is referred to death of conceptuses from day 43 to term. The consequences following a death of a foetus are:

(a) *Complete resorption*: This may occur if the death occurs very early in foetal life. Some of the remnants of the dead conceptus may be voided through the vagina, but may go unnoticed.

(b) *Mummification*: This is discussed under pregnancy detection (see Chapter 13).

(c) *Maceration*: This is discussed under pregnancy detection (see Chapter 13).

(d) *Abortion*: Abortion is defined as the expulsion of a live or dead calf, by less than 270 days of gestation. If calf is alive, it may survive no more than 24 hours. This may occur in any animal or herd and an incidence of less than 5% is considered normal, but anything beyond that must be regarded as abnormal.

(e) *Stillbirth*: Stillbirth refers to a delivery of a dead calf at term.

14.3 Abortions

Abortion defined earlier as the expulsion of dead or live foetus (which survive <24 hours) could be the result of wide variety of insults. For descriptive purposes, the insults could be divided into two broad categories: infectious causes and non-infectious causes.

14.3.1 Infectious causes of abortion

This may result in sporadic or enzootic type abortions.

(a) Sporadic (non-specific) abortion

Viral causes	-	Bovine viral diarrhoea
Bacterial and Rickettsial causes	-	Corynebacterium pyogenese, salmonella species, listeria species, chlamydial species and haemophylus species.
Fungal causes		

(b) Enzootic (specific) abortion

Viral causes	-	Infectious pustular vulvo vaginitis/ infectious bovine rhinotracheitis (IPV/IBR or coital exanthema).
Bacterial causes	-	Camphylobactor species and brucella species and haemophylus species.
Protozoal causes	-	Trichomonas species

14.3.1.1 *Brucellosis:*

(a) *Causative organism and transmission*: This disease is caused by *Brucella abortus* and occasionally by *B. militensis* and *B. suis*. The incidence of abortion in an infected herd varies from 5 to 90%, depending on the number of susceptible animals (pregnant animals), rate of transmission and the virulence of the organism. Infected animals usually abort during the last trimester of pregnancy. The normal transmission of the organism is by ingestion of either pasture contaminated with aborted foetal material and uterine discharges, or contaminated water. Venereal transmission from infected bulls through natural service may occur, but is very rare, as the organisms cannot penetrate the intact vaginal epithelium. However, infection through contaminated semen is possible as semen is deposited in the anterior cervix or uterus. Organisms may also gain entry through mucous surfaces, conjunctiva and through lacerations of the skin.

(b) *Pathogenesis:* Following ingestion by a non-pregnant susceptible animal, the organism may multiply in blood (bacteraemia) and subsequently be localised in lymph nodes. During pregnancy, particularly in the last trimester, the organism by preference invades the pregnant uterus; placenta, endometrium and foetus and causes severe inflammation of these tissues. The organisms, subsequently are localised in the supra mammary lymph nodes and udder. In the case of the male, the organisms may cause inflammation of the seminal vesicles, epididymis, ampulae and testes.

In the pregnant uterus, it causes inflammation of the allanto-chorion resulting in necrosis and oedema of the membranes, resulting in death of the foetus. The most outstanding foetal lesion is broncho-pneumonia. Abortion frequently occur after the 5th month of gestation and retained placenta is a common sequelae. Subsequent uterine infection may interfere with the uterine involution process and may lead to reduction in fertility.

Recurrent infection following conception may occur and could result in repeated abortions. A very small percentage (2-3%) of calves born to infected mothers may acquire the infection *in-utero* or *via* colostrum. Calves may remain serologically negative until pregnancy at which time the latent infection may develop into a full blown infection and cause abortion. In some animals, the organisms may be dislodged from the retreat but would not cause abortion. In either case they become serologically positive by this time.

In the bull, the organism may produce orchitis, epididymitis, vesiculitis including inflammation of other accessory glands. This results in impaired libido and impaired fertility. The bull may discharge organisms in the semen

(c) *Diagnosis*: Diagnosis could be based on the history and lesions in aborted foetuses, followed by isolation of the organism. However, there is a wide spectrum of tests for detecting circulating antibodies that have been developed and which have been used in screening programmes as well as for confirming a tentative diagnosis.

(i) *History and macroscopic features of aborted foetuses*: Abortion in late pregnancies, usually in an epizootic form, in a herd previously free of the disease is highly suggestive of the presence of the disease. High incidence of abortion is usually seen in herds already confirmed to be infected, with a high incidence of retained placenta and postpartum metritis. Aborted foetuses would show characteristic lesions; necrosis of curencles, oedema, haemorrhages in the body cavities and broncho-pneumonia.

(ii) *Bacteriological tests:* Attempts could be made to isolate the organisms from the tissues of the suspected aborted foetus and placental membranes. The appropriate foetal tissues are lung, stomach and placental material. Milk from suspected females and semen from males can also be used. A quick test could be done by examining impression smears from tissues of effected cotyledons following staining with basic fuchin (20%) solution, discoloured by 2% acetic acid and then counter stained with methylene blue. The

background material will be bluish, but if the brucella organisms are present, they take on a reddish tint.

(iii) *Serological tests:* The presence of circulating antibodies (IgM and IgG) signals an infection but not immunity, while surface antibodies (IgA) in the epithelium of the genital tract manifests an immune response. Nevertheless, several diagnostic tests aimed at detecting serum antibodies have been used in many screening programmes, but their value in diagnosis is of little use, because of their inability to differentiate between the serum titres resulting from infections and those following vaccination. Commonly used tests are: Rose Bengal test, Serum agglutination test, Mercaptoethanol test, Complement fixation test and Enzyme-linked immunosorbent assay.

(d) *Treatment:* There is no satisfactory treatment for the condition. The infected pregnancies are most likely to end in abortion and the dam recovers spontaneously following an abortion.

(e) *Control*: Control of the infection is primarily achieved by vaccination of the susceptible population and establishing brucella-free herds in endemic areas. There are several types of vaccines: live (e.g. S19) and killed (S45/20) vaccines available for this purpose.

14.3.1.2 *Camphylobactor foetus infection (Vibriosis)*: This is a venereal disease of cattle caused by *Camphylobactor foetus sub spp. venerealis*, formerly known as *Vibrio foetus*. In general, the condition is characterised by infertility as a result of embryonic losses and fertilisation failures. Abortion may occur in a small percentage of cases.

(a) *Incidence*: The general belief is that this condition does not exist in Sri Lanka, but this has not been proved conclusively. The chances of this disease occurring has been reduced by the wide use of artificial insemination.

(b) *Transmission*: The infection is transmitted by coitus by infected bulls or through insemination with infected semen. In the bull, the organisms becomes localised in the epithelium of the penis and does not produce apparent clinical manifestations (asymptomatic carrier). The bull acquires the infection from a severely infected female during a service. Following the introduction of the organism into the genital tract of a susceptible female, the organism goes through an initial multiplication process in the vagina, in the vicinity of the cervix. From there onwards the infection could take one of few courses:

(i) In about 25% of the animals the infection remains confined to the vagina and in these animals, there is good chance of the pregnancy carrying on to term ending in the delivery of a healthy calf.

(ii) In about 25% of the animals, the organism may gain entry into the uterus by day 7 to 14 following initial infection and proceed to invade the uterus and uterine tubes. The fertilised ovum (conceptus) is then destroyed by the organisms resulting in early embryonic death. The

animal will return to oestrus at the next anticipated date. Thus this condition may lead to repeat breeding.

(iii) In other animals, the organism may enter the uterus a little later and cause endometritis. The organism may destroy the foetus at a later stage causing late embryonic death. The dead embryo may be resorbed fully or expelled. In these animals, the uterine infection is very explicit. The infection may subside within 2 to 3 months of foetal death and the animals may return to oestrus at long, irregular inter-oestrous intervals.

(iv) Still in another group of the animal population, the organisms may be able to invade the conceptus at a later date and infect the uterus and embryo. Inflammation of the placenta causes the death of the foetus, which is usually followed by abortion by 4 to 6 months.

From the above description, it is clear that the clinical symptoms and herd history may very widely.

(c) *Diagnosis*: Diagnosis is based on the herd history, signs and results of laboratory tests. Usually there may be a history of low fertility and high incidence of repeat breeding with animals returning to oestrus at irregular but at longer inter-oestrous intervals. There may be evidence of endometritis such as vaginal discharges and abortion of 4 to 6 months old foetuses. In a chronically infected herd, the above symptoms are largely confined to heifers. A tentative diagnosis could be established by careful evaluation of herd history and clinical signs. Confirmation is by isolation of the organism from material, aborted foetal contents, semen from the infected bull and cervical mucus from suspect animals. Demonstration of antibodies (IgA) in cervical mucus has also been used. Demonstration of antibodies in serum is not possible as the immune response is only limited to the reproductive system.

(d) *Treatment*

Female: Infected females are not usually treated. If there are no gross uterine changes, and reinfection is avoided, nearly 75% of cows may recover in a short time. Others may require 2 to 12 months. A small percentage of cows may carry the infection through a normal pregnancy and harbour the organism in the genital tract after parturition. If no reinfection occurs, the herd could be regarded as free from infection after about 2 years. If however, there is evidence of gross damage to the uterus, then to speed up the recovery, intra-uterine treatment with a penicillin and streptomycin combination could be attempted.

Bull: Infected bulls should be treated. Several treatment approaches have been practised. The organism is susceptible to penicillin and streptomycin.

Local application of penicillin (one million units) and streptomycin (2 gm) dissolved in distilled water, emulsified with a suitable oil base (e.g. vegetable oil, 20 - 100 ml) is infused into the preputial sac and retained there for at least 1 hour by bandaging the

preputial orifice. Three such treatments on three consecutive days are usually sufficient. Alternative treatment is the combination of parentral treatment with streptomycin (at the rate of 25mg/kg bwt) and local application of the same (5 gm in aqueous solution). One such treatment is usually sufficient. Alternatively, local application of either polymyxin and neomycin, singly or in combination could also be used.

Another treatment approach is to switch from natural service to artificial insemination. If this is not possible, then the vaccination of the herd bull as well as the female may be considered. Two sub-cutaneous injections of *C. foetus* organisms in Freunds' adjuvant, 4 weeks apart, followed thereafter by annual booster injections have been recommended.

14.3.1.3 *Trichomoniasis*: Bovine trichomoniasis is a venereal disease of world-wide distribution. It is caused by a protozoan organism called *Trichomonas foetus*. The disease is characterised primarily by abortion occurring mainly during the first five months of gestation and by post-breeding pyometra with macerated embryonic parts. The only proven method of natural transmission is through coitus.

(a) *Pathogenesis*: In the bull, trichomonads colonise only in the crypts of the epithelium of the penis and prepuce. Crypts are deeper and more branched in older bulls and hence old bulls become infected more easily than young bulls. The organisms produce neither clear clinical symptoms nor infertility in the bull. The infection does not induce the production of local antibodies. Accordingly, most bulls once infected will remain so for years or for life.

In the female, the course of infection may take different routes causing variable outcomes. The non-immune female could be infected following a natural service or insemination with infected semen. Following natural service, the organisms multiply in the vagina causing a mucopurulent vaginitis from about day 6 after breeding. In primary infections in cows and heifers, the multiplication of organisms remain confined to the vagina for about 3 weeks before it spreads to the uterus.

(i) Following the initial multiplication phase, in about 25% of the females, trichomonads may remain in the vagina without penetrating into the uterus. Gestation and parturition will be normal in these animals.

(ii) In the majority of cows however, the organisms gain entry into the uterus and kill the embryo directly or indirectly causing inflammation of the endometrium. The embryo is resorbed and animals usually return to oestrus 60 to 90 days after the last service. In some instances, the dead embryo in the uterus may undergo purulent degeneration, while the corpus luteum remains functional. This is probably because of failure of secretion of endometrial prostaglandings.

(iii) The third possible outcome may occur in animals in which the organism may infect the placenta and cause abortion between 2 to 7 months of pregnancy.

(b) *Diagnosis*: A tentative diagnosis could be made with careful evaluation of the breeding history and systematic assessment of apparent clinical symptoms. This condition is usually suspected when several cows in the herd appear to return to oestrus after repeated services by the herd bull. In most cows, the inter-oestrous intervals may be irregular and usually are longer than 21 days. Further, a high percentage of repeat breeding cows may show discharges, usually purulent in nature. The tentative diagnosis is confirmed by demonstrating trichomonad organisms in foetal fluid of the aborted foetus or in exudates associated with pyometra. If these fluids are not available, examination of preputial washings from the herd bull must be made.

A sample of preputial washings is obtained by washing of the preputial cavity with 50 ml physiological saline. This can be conveniently done by occluding the preputial opening following infusion, massaging the prepuce and collecting the trapped fluid into a vessel. The washings are centrifuged, the supernatant is discarded and the sediment is examined microscopically. Mobile flagellated organisms can easily be identified under a light microscope. The diagnosis can be further confirmed by culturing the suspected material for trichomonad organisms.

(c) *Treatment*: Infected females develop local immunity which lasts a few months. Surface active antibodies and hormonal effects of successive oestrous cycles are usually sufficient to free the animal from infection completely. The animal regains normal fertility following the clearance of organisms. If however, re-entry of organisms occurs, the animal may develop the infection as the local immunity is of short duration.

Several treatment protocols have been tried and appear to give good recovery rates.

(i) Washing of prepuce with suitable antiprotozoal solution. Trypaflavin has shown to be very effective. The prepuce and penis could be exteriorised following an injection of a suitable tranquilliser. The mucosal surface is washed initially with 0.1% trypaflavin solution for 10 minutes, followed by the application of an ointment containing Bovo-flavin. This treatment however may cause severe irritation and could cause lesions which sometimes leads to complete loss of libido (impotentia).

(ii) Administration of oral antiprotozoal drugs. There are several effective drugs that can be used. Diamidazole compounds have proved to be very effective. For example, Dimetrenidazole at the rate of 25-50 mg/kg daily for 10 days has given good recovery rates. This drug could also be given intravenously at the rate of 10 mg/kg for 5 consecutive days or at the rate of 50-100 mg/kg as a single injection. The drug is dissolved in 10-20% sulphuric acid. However, this may

sometimes disturb homeostasis which may be manifested as ataxia and respiratory distress. Other drugs such as metranidazole at the rate of 75 mg/kg intravenously daily for 3 consecutive days or 10 mg/kg intravenously at 12 hour intervals daily for 2 days have also proved to effective. Prior to this treatment, 3-5 days of antibiotic cover is recommended.

(d) *Prevention*: Trichomoniasis can be prevented by adequate screening, testing and isolation procedures of all new additions to an established herd. The preputial washings can be tested for organisms. Animals with pyometra or any other genital abnormalities must be eliminated from the herd. The remaining animals must then be bred or served only by artificial insemination. If artificial insemination is not possible, then the herd must be divided into exposed and unexposed groups. The unexposed group could be served by an uninfected bull. The exposed group must be regularly re-examined for any abnormalities, treated as described earlier and given a sexual rest period of 3 to 5 months. In the case of bulls, treatment is not recommended and slaughter of infected animals is recommended.

14.3.1.4 *Other causes of infectious abortions*: Abortion could be a sequalae following a wide variety of bacterial, viral, protozoal and fungal infections. Most of the conditions are dealt extensively in text books on Bovine Medicine. Hence a very short description of each condition and specific diagnostic features are given in Table 14.1.

14.3.2 Non-infectious causes of abortion

Non-infectious abortions could occur due to variety of factors which usually cause sporadic type abortions.

Genetic factors	- chromosomal abnormalities
Endocrine deficiencies	- subnormal luteal function
Nutritional deficiencies	- not clearly elucidated
Toxic substances	- nitrates, mycotoxins, oestrogenic compounds
Therapeutic substances	- oestrogenic compounds, corticosteroids, prostaglandins.
Stress	- heat stress, transport stress, pyrexia,. etc.

Table 14.1 Causative agents, symptoms and diagnostic features of conditions causing infectious abortion.

Causative agent	Symptoms	Diagnostic features
Bacterial Causes: *Corynebacterium pyogenese*	One of the commonest causes of abortion. Develops subsequent to a localised infection such as a chronic wound. Organisms attack the pregnant uterus and cause inflammation of the placenta and foetus. Abortion is usually followed by retention of foetal membranes which usually leads to purulent metritis. Animals would become temporarily infertile and some may become permanently infertile if the uterine tubes are involved.	Purulent inflammatory lesions in the placenta and foetus. Organisms may be demonstrated in direct smears from the foetal tissues and placenta. Confirmation by culturing the suspected material
Salmonella Species: *S dublin and S. typhymurium*	In acute septicaemic forms of salmonellosis, the organisms, S. dublin especially attack the pregnant uterus in addition to causing enteritis due to endotoxins and arthritis. The infected foetus would be aborted and the animal may become a carrier which may contaminate the environment.	Abortion in a cow with characteristic symptoms of salmonellosis (high fever, severe watery diarrhoea, arthritis) is suggestive of salmonella abortion. Confirmation is by demonstration of organisms from suspected material.
Listeria organisms: *L. monocytogenosa*	Initial septicaemia followed by localisation of the organisms in several body tissues. Organisms can cause meningitis and encephalitis (circling disease). In pregnant animals, it attacks the pregnant uterus causing placentitis. In the foetus it mainly attacks the central nervous system. Abortion usually occurs in mid-gestation.	Diagnosis is only possible with the help of bacteriological isolation. Lesions in the brain could be demonstrated in histological sections.
Leptospira Species: *L. pomona, L canicola, L. icterohaemorrhagica and L. grippotyphosa.*	Commonest source of organisms are rodents but symptomless cattle could act as sources of infection as they excrete organisms through urine. Oral infection is the commonest form but is also possible through the respiratory tract, skin and reproductive tract. Following bacteraemia the pathogenic organisms are attracted to the uterus but this usually occurs in the period between 6 to 9 months of pregnancy. The organism attacks both the foetus and placenta. In the placenta, it damages the endothelial	High fever and haemorrhagic milk for a few days. Confirmation by serological examination of dam's blood and by demonstrating the rise in titre of specific antibodies in samples taken 10 days apart. Separation of cattle from pigs, elimination of rodents and treatment of exposed cattle with a suitable antibiotic have been recommended as a

	cells of placental capillaries. This causes foetal death and abortion or delivery of a weak or dead calf at term. Retention of placenta is a common sequelae leading the postpartum metritis.	means of controlling the disease. Double vaccination using isolated strains (polyvalent vaccine) at 2 week intervals would result in establishing solid immunity for at least 6 months.
Heamophilus species: *H. somnus*	This is a recently diagnosed condition. A coccobacillus is responsible for the sleeper syndrome disease. The organism is especially pathogenic to calves and results in septicaemia, followed by pneumonia, arthritis and thromboembolic meningo-encephalitis. In bulls, haemophilus can cause purulent inflammation of the genital organs. In pregnant cows, it could cause inflammation of the foetus and placenta, which may lead to abortion, still births and retained placenta, usually followed by postpartum metritis.	Confirmation only by isolation of the organism.
Rickettsia species: *R. cowdriarumin-antrium and R. phagocytophilia*	Organisms are transmitted through ticks and often through contaminated needles. Virulent organism invades neutrophils and monocytes. In near term pregnant cows, rickettsia may infect the uterus and foetus, causing placentitis which result in abortion and still birth at term.	Rickettsial bodies (round bodies of 0.5 μm in size) in polymorphs. Exposed cows could be treated with a suitable antibiotic.
Chlamydial species	Following infection (oral, aerosol or by ticks), a brief septicaemia ends with organisms settling in the pregnant uterus. Organism attacks the foetus causing inflammatory lesions in the liver, oedema and haemorrhages in various tissues and exudation into the abdominal cavity. Abortion is seen usually from 3 to 9 months of pregnancy. If the foetus survives till birth, the calves look markedly underdeveloped with enlarged lymph nodes. Aborted foetuses may show oedema of subcutaneous tissue, ascitis erythema over the flank and abdomen, petechial haemorrhages in subcutaneous tissues and oral cavity and enlarged lymph nodes.	Characteristic macroscopic lesions may help to establish a tentative diagnosis. Confirmation is by bacteriological examination. Antibiotic treatment of other exposed cows and if cattle are reared along with sheep, separation from sheep from cattle is recommended as preventive measures.

Mycoplasma species: *M. bovis and* *M. bovigenitalium.*	Mycoplasma cause a complex pathological syndrome affecting udder, lungs, joints and in the male – genital organs. Recently, it was found to be associated with sporadic abortion in cows. The establishment of a link between abortion with mycoplasma is difficult because of difficulties in differentiating pathogenic strains from non-pathogenic ones. Organisms appears to inflame the pregnant uterus and cause lesions in the placenta and foetus.	Abortions in a herd with animals showing suggestive signs of mycoplasmosis - pneumonia and joint ill in calves, mastitis which fail to respond to standard antibiotic treatment etc. Confirmation by isolating mycoplasma from suspected material.
Viral causes *Mucosal disease*	Disease could vary from an acute to a chronic form and is characterised by pyrexia. The typical form is characterised by erosion of the alimentary tract, diarrhoea and the appearance of discrete areas of necrosis in the buccal mucosa. About 25% of the pregnant animals infected with this condition, may end up in abortion. Virus attacks the pregnant uterus and it is particularly pathogenic to foetuses of 2 to 4 months of age. In the foetus it preferentially attacks the cerebellum causing micropthalmia and cataract. When older foetuses are infected, most of the surviving foetuses are born weak.	History of mucosal disease in the herd. Appearance of characteristic lesions in the foetus. In weak suspected calves. demonstration of antibodies, prior to uptake of colostrum is a confirmatory test.
Fungal causes *Aspergillus or Mucoraceous species*	Fungi are usually ingested by eating mouldy feed. Following a short septicaemic phase, in pregnant animals the organisms are attracted to the pregnant uterus and causes necrotic placentitis resulting in disturbances in the placental circulation. Abortion usually between 7 to 9 months of gestation. Retained placenta is a common sequalae and the expelled placental parts would show large areas of necrotic patches in the caruncles areas. Fungi usually disappear from the uterus by 3 weeks postpartum.	Macroscopic lesions of the foetus and placenta. Confirmation by demonstration of fungi in suspected contents and by histology.

14.3.2.1 *Toxins*: Toxins of bacterial or tissue origin could be detrimental to foetal survival. This is particularly so in the second half of gestation. One of the commonest types of toxin are nitrates. Nitrates are found in some grasses and these are transformed into nitrites by the bacterial activity in the rumen and are rapidly absorbed from the rumen into blood. Nitrates change haemoglobin into methaemoglobin which disturbs transport and uptake of oxygen in the dam as well as in the foetus. This may cause foetal death and abortion. In the dam, ingested grass or hay with a high content of nitrates may show characteristic signs of nitrate toxicity, muscular tremor, weakness, staggering gait, severe cyanosis with blanching of the mucosa. A weak pulse with terminal convulsions rapidly followed by death has been observed in acute cases. In chronic cases, abortion is a common outcome in pregnant dams.

14.3.2.2 *Trauma and stress*: All severe forms of injury and stress can endanger advanced pregnancies. Examples of these are haemorrhage, pain, fever and stress of surgery. These factors are believed to cause neurogenic hyperstimulation of the uterus and sudden cooling of the peritoneum and bowels. Under these conditions, inactivation of the corpus luteum may occur presumably resulting from secretion of prostaglandins. Some of these threatened pregnancies could be rescued by progesterone treatment. Medroxyprogesterone acetate at the rate of 4 mg per day orally, until 265-270 days of pregnancy has proved to be successful.

14.3.2.3 *Allergy, drugs and vaccinations*: Therapeutic administration of oestrogens, prostaglandings and glucocorticoids have been the commonest causes of induced abortion. All forms of treatments are believed to cause inactivation of the corpus luteum and hence terminate the pregnancy. In addition, tranquillisers such as xylazine hydrochloride (Rompun) could increase uterine contractions and may lead to premature expulsion of the foetus.

14. 4 Other injuries or disorders during pregnancy

14.4.1 Vagino-cervical prolapse

This implies the protrusion of the vagina and cervix through the vulva. The severity of the condition varies from slight intermittent protrusion of the floor of the vagina to severe permanent protrusion of the whole vagina with a part of the cervix.

14.4.1.1. *Causes*: Prolapse is essentially due to weakness of the constrictor muscle of vestibule and vulva and perhaps to stretching of the suspensary ligaments of the genital tract. Several factors may predispose the animal to this condition: (1) factors of genetic origin where some animals and breeds are more predisposed to the condition, e.g. Hereford and Charolais cattle and Nili Ravi buffaloes; (2) obesity which results in extensive disposition of fat in retroperitoneal region; (3) rising levels of oestrogens, particularly in late gestation causing relaxation of pelvic ligaments and are also associated with increased intra-abdominal pressure; (4) constipation and (5) excessive feeding of roughage which results in an increase intra-abdominal pressure. Usually it is a self-perpetuating condition where irritation associated with slight protrusion of the organ may gradually increase abdominal straining resulting progressive increase of frequency and extent of the eversion.

14.4.1.2 *Treatment and prognosis*: A mild degree of protrusion a few weeks before calving is of little consequence. In a more severe prolapse, especially if it occurs 6 or more weeks before term, treatment must be attempted. Failure to do so will result in the breakdown of the cervical mucus seal, bacterial invasion of the uterus and consequent foetal death and abortion.

The main aim of treatment is to retain the prolapsed tissue until the cow calves when, the problem would usually be resolved on its own. The recommended procedure is as follows:

(i) Caudal epidural analgesia to abolish straining.
(ii) Cleaning of the prolapsed organ with suitable antiseptic solution, drying, followed by application of a suitable lubricant.
(iii) Replacing the well cleaned and lubricated organ into the pelvic cavity.
(iv) Placing vulval sutures to retain the organ in place. Several types of sutures such as simple mattress sutures or perivulval subcutaneous sutures have been recommended.

The temporary sutures must be removed as the animal approaches labour.

14.4.2 Uterine torsion

This refers to the torsion of the gravid uterus along its longitudinal axis. Most cases of uterine torsion occur at the time of calving and a small number of animals develop torsion during late pregnancy. It is likely that clinical signs will be manifested only when torsion exceeds 180 degrees about the longitudinal axis. The affected cow will show signs of abdominal pain or discomfort and an be elevated pulse rate.

Treatment and prognosis: Diagnosis could be established by vaginal and rectal palpation. The condition could be corrected by rolling the cow or *via* laparotomy (see Chapter 13, section 13.4.3).

14.4.3 Uterine rupture

This may occur spontaneously for no apparent reason or as a consequence of uterine torsion. The foetus may die or in some cases, when the umbilicus and placenta are intact, it will develop into an ectopic pregnancy.

14.4.4 Hydrops amnii and hydrops allantois

The amniotic and allantoic sacs which are fluid filled organs provide vital functions to foetal development and survival. It protects the foetus from mechanical shock and from possible infections and also act as a reservoir for excretion. At term, the volume of fluid in the amnion and allantois is about 2-3 litres and 9-10 litres, respectively. Hydrops amnii and hydrops allantois refer to an excess accumulation of fluid respectively in the amnion and allantois. Hydrops allantosis is by far the most common condition. In mild cases, the condition may go unnoticed. However, in severe forms of accumulation, the volume of fluid may reach 100 litres in hydrops amnii and 250

litres in hydrops allantois.

14.4.4.1 *Clinical signs and diagnosis*: The excess distension of the abdomen in the last trimester of gestation and reduced appetite are two noticeable signs. Animal reduces the food intake as the rumen volume is compromised by the enlarging uterus. Cows may show difficulty in walking and if the volume is too large, the cow may become recumbent. Rectal examination will reveal a greatly distended uterus.

14.4.4.2 *Treatment and prognosis*: Treatment is not usually recommended unless the cow is valuable. Slaughter of the cow is the preferred choice. If the cow is valuable, induction of parturition with corticosteroids could be attempted. The alternative treatment is the removal of the foetus by laparotomy. However, precautions must be taken to avoid hypovolumic shock, by emptying the fluid slowly over a 30-45 minutes period following the exposure of the uterus through laparotomy. .

14.5 Disorders around pueperium

Injuries and disorders during puerperium have profound affects on the future breeding life of the animal. The following conditions are common: (i) laceration of vulva and vagina; (ii) contusions of the genital tract; (iii) haematoma; (iv) peripheral nerve damage; (v) uterine tears; (vi) placental retention; (vii) uterine prolapse; (viii) endometritis; (ix) metritis and (x) pyometra.

14.5.1 Laceration of vulva and vagina

These occur most frequently following dystocia and are associated with rough obstetrical manipulation.

14.5.1.1 *Vaginal laceration*: This could occur if traction is used without adequate lubrication and also when extension of limbs is attempted without adequate retropulsion. The lesions are not externally visible but the herdsman will observe signs of pelvic discomfort with frequent straining and voiding of fluid exudate. The lesions become infected with opportunistic pathogens.

Treatment: Epidural analgesia could be induced to reduce straining and the infection could be treated with application of suitable an emolient (softening) cream and if necessary treating with systemic antibiotic.

14.5.1.2 *Vulval laceration*: This frequently results in an involvement of the whole perineum region. It could arise when there is foeto-maternal disproportion or vulval strictors or stenosis or when excessive traction is applied before the vulva has relaxed adequately. Different gradations of laceration could occur: *first degree laceration* - superficial laceration involving the mucosa and skin; *second degree laceration* - laceration involving deeper layers of tissue such as the constrictor muscle of vulva and *third degree laceraion* – laceration involving the vaginal wall, anal sphinctor and rectum. This usually results in the creation of a cloaca or fistula.

(a) *Treatment*: Varies with degree of laceration. Usually first degree laceration and second degree laceration would require simple treatment such as suturing of the damaged skin and mucosa and the application of a suitable antiseptic in suitable form. Third degree laceration is difficult to correct and require special surgical correction. In this condition, treatment is delayed until the lesions recover through scar tissue formation. Antibiotic treatment may be required to facilitate the recovery process. By about 6 weeks, tissue repair may be complete and then attempts must be made to repair the damaged parts by surgery. Two methods have been described in standard text books - Gotze's method and Aanes's method. (Refer Handbook of Veterinary Obstetrics - Chap. 13).

(b) *Prevention*: Vaginal and vulval laceration could be prevented by resorting to the technique called episiotomy which involves making an incision (following epidural analgesia) on the vulval opening. The incision is directed in a dorsolateral direction to avoid accidental extension of the incision towards the anus.

14.5.2 Contusions and tears of the genital tract

These are very common outcomes following extensive manipulation of the genital tract in handling a dystocia. Mild contusions such as haematoma may be of little significance but extensive contusions may damage the peripheral nerves - gluteal and obsturator nerves - which may result in paralysis and incordination of hind quarters.

14.5.2.1 *Haematoma*: They are usually associated with rough obstetrical handling, though it could also occur following a normal delivery. If the haematoma is large and occurs before the extraction of the calf, it should be drained through a needle before attempting to extract the calf. If this has occurred after the extraction or delivery, then it is best allow it to heal naturally, as drainage may sometimes lead to abscess formation.

14.5.2.2 *Peripheral nerve damage*: Peripheral nerves (obsturator and gluteal nerves) could be damaged during an assisted delivery. This could also occur during heavy pregnancy due to pressure exerted by the foetus. The nerve damage could be unilateral or bilateral. Obsturator nerves supply the adductor muscles of the hind limb while the gluteal nerve supplies muscles surrounding the pelvis and hind limb. In either case, the cow becomes recumbent and it would be difficult to make it to stand. Any attempt to make it to stand may cause rupture of the round ligament resulting in the dislocation of the hip. Treatment is difficult and recovery also very slow. Some cows may respond to good nursing, such as the provision of plenty of deep bedding and frequent exercise of hind limbs and rolling to improve circulation to hind quarters. Hoisting the cow with slings could be very useful, but care must be taken to avoid excessive pressure build up on the diaphragm.

14.5.2.3 *Uterine tears*: This is also a possible outcome of obstetrical manipulation. Suturing is nearly impossible through vagina. The most conservative treatment is to induce rapid uterine involution and this could be attempted by administering oxytocin at frequent intervals. Surgical correction could be attempted through laparotomy, in cases where the dam is a valuable animal.

14.5.3 Placental retention

The placenta is usually expelled within 6 to 12 hours following the delivery of the calf. Delay in expulsion of the placenta beyond this normal time period is considered as a placental retention.

14.5.3.1 *Incidence*: The incidence of retention of placental membranes could range between 3% to 12% in dairy cattle. It is much lower in beef cattle and also in zebu cattle and buffaloes. The incidence is higher following dystocia, caesarean operation, foetotomy, over distention of uterus, uterine inertia, premature delivery, abortion and also following induced parturition. In Sri Lanka, there are no reports of incidence of placental retention, but the empirical evidence suggests that the incidence is high in Bos taurine cattle and their crosses. The incidence has been reported to be high in herds infected with Brucella.

14.5.3.2 *Causes:* Placental retention could be due to one or more of three basic defects: (i) detachment failure following calving; (ii) failure of expulsive forces due to inertia of the uterus and (iii) mechanical blockage in the expulsive pathway. Before the placenta is expelled, the microvilli must be fully detached from maternal crypts. In the natural delivery process, the detachment of the placenta is a result of the process of maturation which commences in the placenta few days before the onset of parturition. During the maturation phase, there is a significant reduction in the number of epithelial cells lining the maternal crypts. There is also an increase in macrophage activity in the region of microvilli. These and other changes occurring a few days prior to parturition is believed to be mediated by hormones, the very same hormones which are involved in parturition, corticosteroids, oestrogens and prostaglandins. Therefore, it is very conceivable that any disturbance in the endocrine process of parturition will lead to a high incidence of placental retention. Other causes of detachment failure are inflammation in the placental villi and oedema, where the latter condition is a common sequelae following a caesarean operation. Expulsive forces may become defective following the atony due to primary or secondary causes. Finally, the placenta may be trapped mechanically along the cervix and vagina in conditions where the uterus and cervix undergo rapid involution before the expulsion process is completed.

14.5.3.3 *Clinical signs*:. The clinical signs of placental retention could vary from a simple retention of placental membranes to septic metritis.

(a) *In early cases of retained placenta*, the obvious sign within the first 48 hours, is the presence of foetal membranes protruding from the vulva. But in some cases, there may not be any visible signs of foetal membrane parts, as the whole placenta may be trapped inside the uterus. This condition may go unnoticed until the animal begins to show an abnormal uterine discharge or emanates a foul odour, characteristic of degenerating placental membranes. The other signs include vulval swelling, oedema and excessive straining by the cow.

(b) *In delayed cases of retained placenta with septic metritis*, this condition is noticed few days after the parturition. The placental retention may have gone unnoticed by the herdsman. There may be a foetid vulval discharge, characteristic odour and the animal

may show signs of septicaemia and toxaemia - high temperature, diarrhoea, anorexia, dehydration and general malaise.

14.5.3.4 *Treatment*: Treatment methods for retained placenta have undergone very drastic changes over the last few years. The description given here may be used as a guideline by the attending clinician, who could decide on the best course of action to be taken, depending on the duration of the condition and clinical findings.

14.5.3.5 *Manual removal*: This was the method of choice widely used until recently. However, several clinical investigative reports show that manual removal usually leads to added trauma to very fragile uterine tissues and cause retention of thousands of villi entrapped in crypts. These could subsequently act as inflammatory loci eventually leading to metritis and sometimes even to abscess formation. The systemic reaction may be greater with the absorption of toxins following the trauma created during manipulation. The available evidence proves that this was indeed the case in many instances. Further, forced manual removal has been shown to considerably delay the involution of uterus. On the other hand, the continuous discharge of debris from the vulva with a putrid smell is not a condition the herdsman would wish to see. The attending clinician has to make a decision considering all these facts.

Therefore, the following very pragmatic approach could be recommended:

(a) Following aseptic procedures, perform a trial unbuttoning of placental membranes. If there is no fresh bleeding and the placental membranes could be easily removed, the recommendation is to remove the retained placenta as much as possible and leave the rest inside by severing the detached parts from the rest.

(b) Following the manual removal of the detached and easily removable debris of foetal membranes, irrigate the uterus with normal saline or any other suitable solution (e.g. mild antiseptic solution or sterile water) and infuse a suitable antibiotic preparation. Numerous antibiotic preparations have been used. The choice of the antibiotic very much depends on a prior knowledge of the bacterial flora of such conditions. In the absence of such prior knowledge, the clinician would be compelled to use a broad spectrum antibiotic preparation, preferably as a douche or as oblets. Further, attention must be paid to select an antibiotic which would be effective under anaerobic conditions and also effective in the presence of pus-forming bacteria. Note that these two conditions exclude the use of sulpha drugs and streptomycin in the treatment of retained placenta and postpartum metritis. The frequency of intra-uterine antibiotic treatment has also been a matter of controversy. Some argue that more frequent infusion of antibiotics may completely destroy the bacterial flora which is required for the digestion of the retained material. Further, it will arrest any macrophagic activity, which is a part of the uterine defence mechanism thus predisposing the uterus to inflammation. On the other hand, others argue that the extent of the inflammatory reaction is vast and the putrefying material inside the uterus is large. In such circumstance, the inflammatory reaction must be curtailed with suitable antibiotic treatment. The need of a parenteral antibiotic must also to be considered. If the animal does not show any systemic reaction such as anorexia, drop in milk yield and fever, then parenteral antibiotics cannot be justified. However, if the

animal shows systemic signs, the clinician has to consider the parenteral administration of an antibiotic. Again the choice and the duration of antibiotic treatment are matters that the clinician has to decide based on an evaluation of the current condition as well as on the progress. As such re-examination of the animal within a day or two is essential.

14.5.4 Prolapse of the uterus

Eversion of the uterus following calving is referred to as uterine prolapse. In most instances, this occurs within 4 hours of calving. There are several predisposing conditions for uterine prolapse: (i) old age where the incidence is high; (ii) hypocalcaemia, which may cause arrhythmic uterine contractions; (iii) following dystocia, where forceful traction has been applied; (iv) following the retention of the foetal membrane which may cause severe straining and (v) in cows that are recumbent, when hind quarters lie at lower level than the fore quarters.

14.5.4.1 *Clinical signs*: Clinical signs are very obvious. The condition must always be treated as soon as possible, as any delay in correcting the abnormality will result in the loss of viability of the organ. Shock, haemorrhage and thromboembolisms have been reported to be common complications following prolapse, particularly in protracted cases.

14.5.4.2 *Treatment*: This involves cleaning of the averted organ and replacing it with utmost care in the pelvic cavity through the vulval opening. The recommended procedure is as follows:

(a) Before proceeding with treatment, the general health condition of the cow must be evaluated to institute urgent treatment, if warranted. If the cow is showing signs of acute hypocalcaemia, this condition must be treated before any attempt is made to rectify the prolapse. If the cow is in a recumbent position, turn the position of the cow into a sternal recumbency. If the cow is in a standing position, raising the everted organ to or above the level of pubis may help to relieve the pressure on the bladder and to induce urination. Usually the urethra is twisted and the bladder is compressed and hence micturition does not occur. This will also help the clinician in the subsequent step as there will be no resistance from the bladder, when the organ is being replaced into the pelvis.

(b) Employ caudal epidural analgesia to reduce straining. Straining will obstruct the insertion of the everted uterus.

(c) Clean the everted organ thoroughly with a suitable antiseptic solution or normal saline or sterile water. Remove all contaminated material and if any placental material still remains attached, cut off the loose parts and leave the rest. If there are tears, repair those with simple sutures using absorbable suture material.

(d) Commence the insertion of everted organ, starting from the parts nearest to the vulva. At the beginning it may appear difficult but gentle pursuasion will

eventually succeed in getting the organ into the pelvic position. An assistant may hold the organ in position while the clinician is working gently on it.

(e) Once the organ is placed in pelvic position, insert the clean, cuffed hand deep into the uterus and push it cranially and ventrally to ensure that the organ has completely regained its normal position and shape. The organ must then be flushed with a suitable antibiotic - preferably a broad spectrum antibiotic in oblet or powder form, ensuring thorough distribution within the entire uterine cavity.

(f) To avoid a recurrence and to keep the organ in pelvic position, place several sutures across the vulva. There are several types of vulval sutures - purse-string or simple mattress sutures that have been recommended. In order to avoid a recurrence, it may be desirable to maintain epidural analgesia, for a few more hours. For this purpose, re-examination of the animal after 12-24 hours may be highly desirable.

14.6 Disorders during postpartum period

14.6.1 Postpartum metritis

Inflammation of the endometrial and muscular layers of the postpartum uterus is referred as postpartum metritis. This condition is often a secondary complication of parturition such as dystocia and retained placenta. The symptoms are manifested at varying periods from the time of parturition. The condition is usually characterised by an abnormal, foul smelling discharge from the vagina which is often expelled through vulva. The discharges may become dry outside and form crusts around the perineum. This is different from the normal discharge that occurs following parturition which is called uterine lochia. It is reddish brown in colour and mixed with mucoid and devoid of offensive smell. This last for about 2 weeks.

14.6.1.1 *Causes*: Contamination of uterus at parturition may occur when the delivery of the young occurs under unhygienic conditions and usually as a sequelae following conditions such as dystocia, prolapsed of the uterus and retained placenta.

14.6.1.2 *Clinical manifestations*: Clinically the condition exists or is manifested in several forms: acute to subacute to chronic metritis.

(a) Acute septic metritis: Acute septic metritis is usually restricted to the first 5 to 10 days postpartum. Acute metritis is characterised by signs of septicaemia (high fever, diarrhoea, anorexia, reduction in milk yield, elevated pulse and respiratory rates and possibly dehydration). In advanced cases, the animal may show subnormal temperature and may become recumbent. The uterine discharge is usually reddish brown in colour with an extremely offensive smell. Rectal examination will reveal that the uterine wall is thin, atonic and the uterine cavity is filled with fluid.

(b) Subacute metritis: This condition appears later in the postpartum period; i.e. usually about 8 to 14 days postpartum. It is characterised by uterine infection with or without mild septicaemic signs. The usually observed signs are - moderately elevated

temperature, mild anorexia and lowered milk yield. There may be a thick, reddish brown to yellow discharge from the vulva or dried exudate over the perineum region. The vulval lips may be swollen. Rectal examination will reveal a uterus that is still involuting, filled with fluid, a thickened wall (because of oedema) and flaccid to turgid in consistency.

(c) Chronic metritis: Chronic metritis may be an extension of the acute infection into a chronic stage and usually manifested late in the post partum period. There may be scanty vulval discharges usually purulent in nature. Rectal examination may reveal a uterus that has not returned to a non-gravid state completely and the uterine wall may be thick and oedematous and the lumen may contains a volume of fluid. Rectal examination may provoke expulsion straining and hence the expulsion of exudate through vulva.

In all these conditions the common organisms involved are *Corynebatcterium pyogenes, streptococci and staphylococcal species, coliforms and fusobacterium species.*

14.6.1.3 *Treatment*: The following guidelines are provided to help the attending veterinarian to formulate a suitable treatment plan, depending on the nature of the condition.

(i) If excessive manipulation such as uterine emptying and infusions are to be employed, it may be advisable to induce epidural analgesia to prevent abdominal straining.
(ii) If a large volume of fluid is present in the uterus, the removal of exudate would be desirable. However, it must be done with great care as excessive manipulation may lead to increased absorption of toxins from the exudate, trauma and even rupture of the uterus. The standard procedure is to siphon out the exudate through a tube: a soft, clean, flexible tube (e.g. rubber tube) passed transvaginally into the uterus and the open end of the tube is placed at the lowest part of the uterus with the other end fixed to a funnel or fluid receptacle. A suitable irrigation fluid (e.g. mild antiseptic solution or saline or sterile water or boiled but cooled water) is poured into the receptacle and fluid is allowed to flow into the uterus through gravity. Before the fluid is completely emptied, the receptacle is lowered to a point below the level of uterus and fluid is allowed to flow back out of the uterus through the tube into the receptacle. The procedure is repeated several times until the fluid coming out from the uterus no longer contains debris.

(iii) *Antimicrobial treatment:* A suitable substance, either antibiotic or antiseptic to eliminate the microorganism must be employed. Intra-uterine administration of antibiotics usually results in higher concentration of the drug in the uterine tissue and uterine lumen. Adequate levels will be maintained within the uterine cavity over a long period as metabolism of the drug is not rapid. However, this route may not provide adequate concentrations in the deeper tissues of the uterus and also in the oviduct, cervix and vagina. High levels in the tissues may only be achieved by parenteral administration. Therefore, it is recommended that the antibiotic is administered *via*

both routes, particularly if the animal shows septicaemic signs and if the involvement of deeper tissues of the uterus and other parts of the reproductive tract is suspected.

(iv) *Choice of antibiotic*: Ideally this depends on the results of antibiotic sensitivity tests. If this is not available, the use of a broad-spectrum antibiotic or a mixture of antibiotics is recommended.

For intrauterine administration: The following drugs have often been used.

Procaine penicillin	- 4 million units
Chloramphenicol	- 2 gms
Oxytetracyclines	- 1 gm
Neomycin	- 1-2 gms
Polymyxin	- 3 million units

The drug chosen from the above list is dissolved in a suitable volume of distilled water (i.e. depending on the volume of the uterus) and infused into the uterus, through a suitable apparatus (e.g. syringe fitted with a rubber tube). The uterus should be thoroughly massaged gently following the infusion to ensure even distribution within the uterus.

This treatment could be combined with parenteral antibiotic treatment. Oxytertacyclines (5-10 mg/kg) through intra-uterine route and procaine penicillins (10,000 -20000 IU/Kg) the parentally have been commonly used. This therapy has given successful results even though it deviates from the guidelines given for antibiotic treatment (i.e. not to combine a bacteriocidal drug with a bacteriostatic drug). Both forms of treatment need to be repeated at frequent intervals for 3 to 4 days while monitoring the progress.

Supportive treatments: Drugs to facilitate uterine contractions have been used. Oxytocin with or without prior treatment with oestrogen and prostaglandins have been used. Prostaglandins are more suitable, in more chronic cases, particularly with a CL tissue in the ovary. Oestrogens also have been used. The rationale behind oestrogen treatment is to increase the uterine contractility, increase blood supply and facilitate uterine defence mechanisms. However, this treatment may also enhance the toxin absorption from the uterus, thus causing toxaemia.

(v) *Prognosis*: Recovery rate after early treatment is high and fertility of the animal could be restored. However, in animals with extremely inflamed uterus, recovery may be doubtful and future reproductive life may compromised.

14.7 Endometritis

This is an inflammation of the endometrium. However, in practice it is difficult to know whether the deeper layers of the uterine wall are involved or not. Hence the term endometritis and chronic metritis are used indiscriminately to describe the chronic inflammation of the uterus.

14.7.1 *Causes*: Endometritis, in general is a chronic condition which usually occurs in cows that have had retained placenta or acute metritis and are presumed to have recovered from the treatment. However, this could also occur in cows that have not had any complication at parturition or during the postpartum period. This may have arisen either due to contamination of the genital organs at parturition or contamination of the uterus at the time of insemination, or due to recurrent bacterial diseases. The common organisms that act as the primary pathogens are *Mycobacterium tuberculosis, Brucella abortus, Camphylobactor foetus, Trichomonas foetus and IBR-IPV* organisms or secondary pathogens such as *Corynebacterium pyogenes, streptococcal and staphylococcal* organisms, *E-coli and fusibactor* organism.

14.7.2 *Clinical findings*: Based on the symptoms which depends on the degree of tissue involvement, the condition could be categorised into *first degree, second degree or third degree endometritis*. In first degree endometritis, the reproductive tract of the animal appears normal but the animal has a history of a failure to conceive after repeated services. Visible external signs or appreciable changes in the reproductive organs are not evident in most instances on rectal examination. In second degree endometritis, there may be a slight vulval discharge and appreciable changes such as oedema of the uterus wall (thick and turgid uterine wall) and a small volume of exudate in the uterine lumen may be evident when examined per rectally. In *third-degree endometritis* there may be a visible mucopurulent discharge and appreciable changes (thick uterine wall, fluid, atony of the wall etc) on per rectal examination indicating a uterine pathology. In all these conditions, the animal may not show any sign of ill health such as alteration in body temperature, feed intake or milk production. The vulval discharge usually shows mucopurulent material of varying consistency. Vaginal examination sometimes may reveal congestion of the cervix and the anterior part of the vagina. The ovaries may be normal and most cases will show ovarian cyclicity.

14.7.3 *Treatment*: There has been different treatment protocols for dealing with this condition, often producing mixed results. The following guidelines may be found to be useful in deciding on a treatment protocol by an attending veterinarian.

The uterus has its own defence mechanism. The periodic exposure of the uterine tissue to progesterone followed by oestrogen appears to play a vital role in maintaining the natural defence mechanism. The defence mechanism is more efficient in the non-pregnant cow when the phases were dominated respectively by oestrogen and progesterone succeeding one another in physiological order. Oestrogen enhances the uterine defence mechanisms by promoting the discharge of uterine exudate because of the increase in smooth muscle contractility and by increasing the permeability of the epithelium to microorganism, so that leucocytosis and phagocytosis become stimulated which subsequently leads to production of local antibodies. However, when oestrogen domination persists for an unnaturally long period as in cystic ovarian disease conditions, all these beneficial effects fail and the uterus by contrast becomes abnormally susceptible to infection. This natural defence mechanism could be taken into consideration and the following guidelines are of value when designing a treatment plan.

14.7.3.1 *Prostaglandin therapy:* If the ovary bears a CL, administration of $PGF_{2\alpha}$ is recommended. The rationale behind this treatment is to induce lysis of the CL and reduce the luteal phase and hasten the return to the oestrogenic phase of the cycle. Prostaglandin is also a potent stimulator of uterine contractility. Both of these effects will enhance the ability of the uterus to eliminate the infection.

14.7.3.2 *Oestrogen therapy:* If these is no CL in the ovary, oestrogen treatment may be considered, to create an oestrogenic phase by giving oestrogen exogenously. This treatment is usually recommended in combination with suitable intrauterine antibiotic therapy.

14.7.3.3 *Intrauterine infusion of antibiotics:* This treatment is the most commonly used and also the oldest treatment procedure. Many antibiotic preparations have been used. The most commonly used ones are - tetracyclines (1-2 gms), penicillins (1-2 million units), chloramphenicol (1-2 gm), polymixyn (2-3 million units), etc. The antibiotic chosen is dissolved in a suitable volume of distilled water or saline (depending on the volume of uterus, usually in 5-10 ml) and infused into the uterus which is then followed by thorough massage to ensure even distribution of the drug in both uterine horns.

14.7.3.4 *Intrauterine irrigation with Lugol's iodine solution:* This was the oldest form of treatment, but discredited in the past because of the claim that it may cauterise the uterine endometrium beyond regression. However, a mild solution (i.e. 1-2% solution) may act as an irritant which may cause production of endogenous prostaglandin which will then bring about the desirable action; i.e. demise of CL, induce the oestrogenic phase and increase uterine contractility which will facilitate the recovery process. Besides precipitating uterine prostaglandin production, Lugol's iodine itself will act as a mild antiseptic and exert a cleansing effect on the uterine endometrium.

14.7.4 *Prognosis:* The condition usually responds to treatment and animal will regain normal fertility.

14.8 Pyometra

This implies the accumulation of pus in the uterus as a result of uterine infection. The condition is characterised by the absence of oestrous activity with intermittent discharge of mucopurulent material from the uterus (luechorrea).

14.8.1 *Causes:* This may usually arise as an extension of postpartum metritis or it may be precipitated by service naturally or artificially with infected semen or bull harbouring *Trichonomas foetus* or *Camphylobactor foetus* organisms. In the former case, the commonly encountered organisms are *Corynebacterium pyogenese, streptococcal and staphylococcus* organisms, *pseudomonas and pasteurella* organisms.

14.8.2 *Clinical signs:* An abnormal discharge may be commonly observed from the vulva. In most instances, the discharge may be seen on the floor, where the cow has

been lying. Vaginal examination will reveal the congested vestibular, vaginal and cervical mucosa usually with flakes of pus in the vaginal vault. Rectal palpation will reveal an enlarged uterus. Uterus is filled with fluid and the wall is usually thicker than in pregnancy. In some cases there may be a CL in the ovary and invariably this is most likely to be due to a persistent CL.

14.8.3 *Treatment*: Uterus may have been extensively damaged at this stage and hence any form of treatment may not have much beneficial effects in terms of restoring normal fertility. If treatment is considered, it must be aimed at evacuation of uterine contents and controlling the uterine infection. For evacuation of the uterus, $PGF_{2\alpha}$ may be effective. It will not only stimulate uterine contraction, but also bring about the demise of the CL. The choice of antibiotic depends on the antibiotic sensitivity test results, but in the absence of such information, broad spectrum intra-uterine antibiotic treatment is recommended.

14.9 Other reproductive disorders in female

14.9.1 Freemartinism

Freemartin is a sexually deformed female of a heterosexual twin in multiple bovine pregnancy. Freemartinism occurs in about 80% of twin heterosexual bovine pregnancies. The sexual deformities are caused by substances produced by the developing male foetus which cross into the female foetus through a vascular anastomoses which commences development by day 20 to 30 of pregnancy. Two different foetal testicular substances or materials may traverse from the male to female foetus through these anastomoses. One of them is androgen which will stimulate the development of male Wolffian ducts while the other is a non-androgenic polypeptide, called the Mullerian duct inhibitory factor which inhibits Mullerian duct development. Besides these two testicular substances, primordial germ cells from the male (XY bearing cells) migrate through the anastomoses and inoculate the medullary region of the undifferentiated female gonadal tissue. The development of female components of the foetal genital organs would be derailed and the XY bearing cells may differentiate into testicular tissue and hence cause the development of an ovo-testes.

The sexual deformities of the foetus is characterised by atrophic, inactive ovaries and variable deformities of the vagina and frequently a hypertrophied seminal vesicles and clitoris. The general phenotypic appearance of the freemartin is that of a female but may behave like a male. The pelvic region, is usually narrow and upon examination of the vulval region the hypertrophied clitoris and blind vaginal vault may be evident. Rectal examination may reveal hypertrophied seminal vesicles and missing segments of some parts of the reproductive tract. Upon examination of chromosomal complement (karyotyping), the animal may show a chiamerism (i.e. presence of XX and XY populations of cells in the same individual).

14.9.2 Inactive ovaries

It could be either congenital or acquired.

14.9.2.1 *Congenital ovarian inactivity:* This is believed to be caused by a recessive gene in most instances and in the past it was believed that it may occur only in white breeds of cattle. A deficient inoculation of the embryonic gonadal ridge with primordial germ cells may result in an unsatisfactory activation of the cortex which remains hypoplastic and is largely or totally devoid of primordial follicles. Hypoplastic ovary will not be able to initiate folliculogenesis, steroid production and ovulation. As these conditions could be transmitted to next generation, these animals must be disposed of from the breeding stock. Because this condition was first detected in animals with white skin coats, it was usually referred as white-heifer disease. However, this could occur in any irrespective of coat colour.

14.9.2.2 *Acquired ovarian hypotrophy (anoestrus):* This is usually followed by calving. Animals following calving may not resume ovarian activity within the stipulated time (i.e. within 45 to 60 days) and hence usually referred as postpartum anoestrus. In this condition, the ovarian architecture is normal but there is no follicular development and ovulation. The aetiology and pathology is complex. Many factors have been shown to contribute to uterine inactivity.

(a) Underfeeding during pre- and post-partum periods
(b) Unfavourable environmental conditions such as dry, hot and humid weather accompanied with scarcity of feed
(c) Suckling by a calf - the suckling act has been shown to be inhibitory to resumption of postpartum ovarian activity.
(d) Chronic disease conditions which results in a drop in feed intake and a marked loss of weight
(e) Any form of stress

14.9.2.3 *Clinical findings:* Animal remains in anoestrus. Per rectal examination may reveal small and smooth ovaries without follicular activity or a CL. The body condition score may be usually less than 2 in a scale of 1 to 5 (1 - emaciated and 5- very obese, see Chapter 28 for more details on Body Condition Scoring System).

14.9.2.4 *Treatment:* The postpartum anoestrus is a reversible disorder and the treatment varies with the underlying cause.

(a) If nutritional deficiencies or underfeeding is suspected as reflected by the poor body condition, correction of the deficiencies must first be attempted. The cow should regain its body condition to at least a score of above 3. The correction involves providing a feeding system which provide adequate amounts of energy, protein, vitamins and minerals (refer Chapter 6 for balancing rations).
(b) If free suckling is practised then the reduction of the suckling stimulus either by complete weaning by day 7 or limited suckling (suckling once or twice a day) must be attempted[1].

[1]Abeygunawardena *et al.* (1996a)

(c) If there is no suckling stimulus and the cow is in good body condition (>2.5 BCS), then stimulation of ovaries with exogenous reproductive hormones must be attempted. There are several hormonal treatment protocols[2].

(i) GnRH: The gonadotrophin releasing hormone has been used widely in conditions with sufficient follicular activity ("moderate" anoestrus) which is presumed to have failed to go beyond latter stages of follicular development (e.g. antral into pre-ovulatory stages). The ideal therapy with GnRH is the pulsatile administration of small doses of GnRH at hourly intervals over 25 to 48 hours. But this is not practically possible and the commonly used method is the use of synthetic GnRH which are long acting (e.g. Bruceralin) given as bolus injections, either one or two injections 12 or 24 hours apart. The response to GnRH therapy could be enhanced by pre-treatment with FSH or PMSG. This combination may also be considered for animals with ovaries without any follicular activity ("deep" anoestrus) as the pre-treatment with PMSG or FSH would stimulate follicular development.

(ii) Progesterone therapy: The rationale behind the use of progestagenic compounds is the make use of the phenomenon called the *pituitary rebounding effect*. It has been shown that high levels of progesterone at the hypothalamic level reduce the amplitude and frequency of GnRH pulses and this will cause the preferential synthesis and storage of LH over synthesis and release. After a period of a few days there will be a build up of a sufficient quantity of LH in the pituitary and upon removal of progesterone, the pituitary rebounds by releasing large quantities of stored LH. This may then act on the ovary and stimulate folliculogenesis, with eventually one follicle reaching the pre-ovulatory stage and subsequently undergoing ovulation. There are several types of progesterone preparations - Progesterone releasing intra-vaginal device (PRID) or progesterone releasing subcutaneous silastic implants (e.g. CRESTAR). The standard protocol is to leave the implant or the coil in situ for 9 to 11 days. Upon removal of the implant, the animals are expected come into oestrus within 36 to 96 hours. The spread could be reduced and the induction rate could be enhanced by giving an injection PMSG (250-500 IU) or GnRH (equivalent of 250 ug of natural GnRH) at the time of implant or coil removal. Usually two services one at 48 and other at 60 hours post-implant removal are recommended to achieve high conception rates.

14.9.3 Cystic ovarian degeneration

This is generally seen in high producing dairy cows, usually after calving. The ovary shows continuous follicular activity but fails to undergo ovulation or atresia (i.e.

[2]Abeygunawardena *et al.* (1996b)

degeneration). Fluid tends to accumulate within the follicle and eventually form cystic structures. The cystic structures go on producing steroids, primarily oestrogen, but a few cystic follicles may undergo partial luteinization (note: luteinization is a result of a degeneration process in the steroidogenesis pathway with the loss of a few rate limiting enzymes) thus producing progesterone in addition to oestrogen. Per-rectal examination will reveal the presence of one or more of large follicles (>2 cm in diameter). Under the influence of oestrogen from the cystic follicles, cows affected with this condition exhibits characteristic signs of oestrogenism and hence herdsmen tend to refer to the condition in the cow as a continuous oestrus (nymphomania).

This condition is more common in dairy animals than in beef animals. In Sri Lanka it is more common in Bos taurine cattle and cross-breds than Bos indicus of exotic or indigenous origin.

14.9.8.1 *Aetiology and pathogenesis:* The aetiology and pathogenesis are not well understood. The available evidence, however implicates the involvement of the entire hypothalamo-pituitary-gonadal axis and also possibly the adrenal gland. There is also evidence to suggest an existence of hereditary predisposition in some animals. Further, there also exist a high correlation between high milk yield and cystic ovarian condition. The highest incidence as reported in animals in the 4th and 5th lactations when highest yields are usually recorded. Cystic follicles are common within few weeks after calving but most of these heal spontaneously by day 50 to 60 postpartum without causing any apparent clinical syndrome. There were also reports implicating exogenous oestrogens in precipitating this condition.

The proposed hypothesis with regard to the pathogenesis of the condition is as follows. The dynamic balance of secretion of hypothalamic releasing hormones (i.e. GnRH and possibly FSRH?) is broken, and as a result there will be an altered release of LH and FSH, thus altering the ratio of FSH:LH in blood in favour of FSH. It is also suggested, based on experimental findings, that the follicles destined to become cystic may carry more FSH receptors than LH receptors (*note- it was mentioned earlier that induction of LH receptors in granulosa cells is a requirement for follicular selection and continuation of the development to the preovulatory stage*). Any deficiency of LH receptors may conceivably result in the failure of follicles to proceed further in follicular development. In cystic animals, it was also noted that the levels of ACTH and cortisol are usually elevated. Whether it is involved in the actual disease process or whether it is responsible for producing symptoms like virilism (male like characteristics) in this condition is not known. Further, animals with cystic ovaries tend to show alterations in sexual behaviour, possible due to alteration of sex behavioural centres under high levels of oestrogen.

Within the cystic follicles, the walls of the enlarging follicles may commence degeneration, the oocyte dies and in a certain percentage of follicles, the cells of the follicle wall may undergo partial luteinization. In these animals high levels of plasma progesterone are usually found and hence these animals may not show oestrogenic signs in contrast to that seen in typical cases. Because of these changes in cystic follicles, many have classified cystic ovarian conditions into two categories: follicular cysts and luteinized cysts.

Follicular cysts: The wall of the cystic follicles are thin and in blood there is more oestrogen present than progesterone (on a ratio basis not on absolute terms). The animals show a recurrence of oestrogenic signs and hence the affected animal is usually referred as nymphomaniac cow.

Luteinized cyst: The follicle wall is still thin in consistency, but in blood there may be higher levels of progesterone and androgens than oestrogens (on a ratio basis). The cow usually will be in anoestrus.

14.9.3.2 *Diagnosis*: Diagnosis could be established on the basis of history - recurrent oestrogenic signs which may be seen in animals at the onset of oestrus at short intervals and showing oestrus-like signs lasting for few days or as a failure of the animal to show oestrous signs (anoestrus like condition). Per-rectal examination may reveal characteristic features of cystic follicles - presence of one or more of large, thin walled follicles, giving a mulberry appearance. Hormone measurements will reveal high oestrogen and/or progesterone in plasma.

14.9.3.3 *Treatment:* If animals are left untreated, cows may remain in continuous excitement and very soon they may become exhausted and reduce feed intake resulting a drop in milk yield and body condition. There are many treatment protocols for dealing with cystic ovarian condition, but they yield varying results. The following guidelines would be useful in deciding on a treatment regime.

(i) If the condition is diagnosed as cystic ovarian degeneration with follicular cysts, then the aim of the treatment would be to cause ovulation or luteinization which would eventually degenerate. For this purpose, the most commonly used hormone is chorionic gonadotrophin hormone (hCG), at the dose rate of 3000 to 5000 IU given intravenously. The alternative treatment is to use GnRH or GnRH analogs at dose rates of 500 µg of natural GnRH or equivalents doses of analogs.

(ii) If the condition is diagnosed as cystic ovarian degeneration with luteinized cysts, then the aim of the treatment is to lyse the luteal tissues. For this purpose the drug of choice is $PGF_{2\alpha}$, at a dose rate depending on the type of analogs.

(iii) The oldest, but not widely advocated method is the manual rupture of the follicle. This may lead to bleeding and sometimes it could lead to adhesion formation and even death of the animal.

14.9.3.4 *Prevention:* As it is believed that there may be some hereditary predisposition, some countries (e.g. Sweden) adopted selection of animals against cystic ovarian condition. Indiscriminate use of oestrogenic compounds for treatment of infertility must be avoided.

14.9.4 Pneumovagina

Tilting of the vaginal opening cranially could occur following traumatic disturbances to vulva, caesarean operation (due to adhesion formation) and also due to old age. As the vaginal vault slants ventrally, there is a tendency to suck air into the vaginal vault. Further, urine tends to get accumulated in the anterior part of the vagina. These conditions, respectively are called pneumovagina and urovagina. The accumulation of urine in the anterior part of the vagina may sometimes lead to the formation of a pouch with continuous accumulation of urine and this could result in inflammation of vagina and cervix. Further, this condition could also lead to cervicitis and sometimes to endometritis. Hence, the condition could lead to depressed fertility, particularly if natural service is practised. With natural service, contamination of semen deposited in the vagina with accumulated urine may result in a reduction in viability of sperms.

There is no satisfactory treatment to improve fertility. As a preventive measure, conditions which may result in trauma to external genital organs and favour adhesion formation must be avoided.

14.9.5 Tumours and carcinomas of the vulva

These conditions may be encountered with cows with unpigmented perineum region. No satisfactory treatment is available hence culling is recommended.

14.9.6 Defective functioning of the fallopian tube

Fallopian tube or oviductal defects may occur following adhesion formation between the fimbria and the ovarian ligaments and ovarian bursa. Its functions could also be affected by inflammatory conditions, usually resulting from extension of uterine infection centripetaly. The oviduct may also become defective due to congenital defects such as in white heifer disease condition, segmental aplasia, etc. Animals having defective oviducts usually exhibit a high degree of repeat breeding - i.e. return to oestrus at regular intervals despite insemination with fertile semen or service by a fertile bull. Rectal examination may sometimes reveal the palpable oviduct (note: under normal conditions oviduct cannot be palpated). This condition cannot be treated easily and could eventually lead to sterility.

Selected references

Abeygunawardena, H., Kuruwita, Y.V. and Perera, B.M.A.O. (1996a). Effects of different suckling regimes on postpartum fertility of buffalo cows and growth and mortality of buffalo calves. *The Role of the Buffalo in Rural Development in Asia*, SAREC/NARESA Press. pp. 321-336.

Abeygunawardena, H., Kuruwita, V.Y. and Perera, B.M.A.O. (1996b). Effects of exogenous hormones on fertility of postpartum anoestrous buffaloes. *The Role of the Buffalo in Rural Development in Asia*, SAREC/NARESA Press. pp. 337-350.

Arthur G.H., Noakes, D.E. and Pearson, H. (1982) *Veterinary Reproduction and Obstetrics* (5th edition). Bailliere and Tindall, London.

CHAPTER 15

Manipulation of Reproduction

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Introduction

Ever since the man commenced rearing animals for food and particularly with commercialisation, reproductive processes have been subjected to manipulation for the benefit of the man. Over the last few decades, spectacular progress has been made in this field. Of the most important manipulative processes, artificial insemination stands out as the most significant development in this regard. As this topic has been dealt separately under Chapter 11, no attempt will be made here to explain artificial breeding in farm animals.

The following topics will be discussed.

1. Induction of oestrus
2. Control of oestrus (oestrous synchronisation)
3. Control of ovulation
4. Super-ovulation and embryo transfer
5. In-vitro fertilisation

15.1 Induction of oestrus

This is one of the potential areas for manipulation. In most production systems, resumption of ovarian activity following parturition is usually delayed due to a wide variety of causes such as poor nutrition, suckling and adverse weather conditions. Therefore, the best form of approach is the removal of the precipitating causes.

In cattle and buffaloes, to achieve an ideal calving interval of 12 to 13 months, the postpartum cow must resume oestrous activity within 40 to 60 days postpartum with eventual conception occurring within 90 to 100 days postpartum. Any delay in resumption of postpartum ovarian activity beyond 60 days will result in long inter-calving intervals, which are unproductive, as this will (i) increase the dry period of the cow, (ii) reduce the total milk yield per lifetime of the cow and (iii) result in fewer progenies per cow per lifetime.

As stated earlier, the most rational treatment or approach to induction of oestrus in anoestrous animals is the removal of the precipitating cause. Therefore, the preferential order of the treatment approaches is (i) adoption of appropriate nutritional management, (ii) removal or reduction of suckling induced inhibition and (iii) as the last resort the hormonal induction of oestrus.

15.1.1 Adoption of appropriate nutritional management

There is a wealth of evidence to link poor nutrition with the delay in resumption of postpartum ovarian activity. Though the exact mechanism and the relative importance

of the deficient nutrients in this context is not well known, in general, deficiencies of energy, protein and macronutrients, such as phosphorus, calcium, sulphur and vitamin A have been associated with prolonged postpartum anoestrus. In addition, the deficiencies of micronutrients such as trace minerals, zinc, cobalt, copper and selenium, and vitamin E have also been implicated as possible causes of anoestrus. The detection of a particular deficiency under field conditions is extremely difficult, but nevertheless, there are some approximations that a clinician could make to assess the nutritional status of an animal. The simplest method is the assessment of the body condition of the animal. Chapter 28 of the Handbook describes a method to assess the nutritional status of animals by estimating the body condition.

If the nutritional status of the animal is poor (BCS < 2), the herdsman must be advised to provide a balanced ration. The chapters 1 through 6 provide the required information in this regard. The final prescription would be the use of a recipe for the provision of a cost-effective balanced ration.

15.1.2 Adoption of an appropriate suckling management practice

Suckling by the calf has been shown to have an inhibitory effect on the resumption of postpartum ovarian activity. A great deal of work has been done to elucidate the underlying reasons why suckling induces postpartum anoestrus. These studies have demonstrated that suckling *per se* as well as the cow-calf interaction are equally inhibitory and that postpartum ovarian function is inhibited by delaying and suppressing the development of the pulsatile gonadotrophin secretory pattern postpartum. How this inhibition is brought about is not certain, but available evidence suggests that it may be due to a mechanism mediated at the level of hypothalamus. Suckling also has been shown to stimulate the secretion of prolactin and cortisol and these two hormones may also act in concert with the hypothalamic mediated inhibitory mechanism. Various studies have shown that weaning at various stages postpartum as well as limited suckling (once or twice a day suckling) have been effective in inducing early resumption of postpartum ovarian activity in dairy animals. As early weaning requires feeding of milk collected from the cow or a suitable replacement, the additional cost involved may not be compensated by the improvement in reproductive efficiency. Therefore, the most economical suckling management practice is to limit suckling. In this method, in both intensively and extensively managed herds, the calf is separated from the mother and allowed to suckle the mother soon after milking for 30 to 40 minutes, once or twice a day, depending on the management system.

15.1.3 Use of exogenous hormones

Various hormonal preparations in different combinations have been used. The response and success rates in terms of induction and conception are highly variable and appear to be influenced by the age and nutritional status of the animal and the season of the year. In animals in poor body condition (BCS < 2), old animals, and during adverse climatic conditions (e.g. drier part of the year) the success rates of both induction and conception are very low.

15.1.3.1 *Use of gonadotrophin releasing hormone:* Our understanding of the hypothalamo-pituitary gonadal axis has been improved tremendously over the last two decades. It is now well established that the driving force for pituitary gonadotrophin secretion originates at the level of hypothalamus and the hypothalamic GnRH is directly responsible for synthesis and release of both FSH and LH. GnRH has been used in different treatment protocols and the more potent and long acting preparations of GnRH are now available in the market. It is also well established that GnRH is released in a pulsatile manner from the hypothalamus and the pituitary gonadotrophins become refractory if continuous infusion of GnRH is attempted. Therefore, the most effective method is the administration of GnRH in a pulsatile manner, but this is not practically feasible. The commonly used treatment method is the bolus injection of GnRH given as a single injection (100 to 500 μg of natural GnRH or equivalent doses of GnRH analogs) or double bolus injections given 12 to 24 hours apart. However, the success of this treatment is related to the status of the ovary (or degree of anoestrus) at the time of treatment. If the ovary shows some follicular activity (referred to as "moderate" anoestrus), this treatment is most likely to be successful as against the situation where the ovary is devoid of any appreciable level of follicular activity (referred to as "deep" anoestrus).

15.1.3.2 *Use of gonadotrophin:* The use of exogenous gonadotrophin (e.g. FSH, PMSG and hCG) in the induction of oestrus was the most common form of treatment a few years ago. These treatments, produce variable results and empirical evidence suggest that the success rate of inducing oestrus and also subsequent conception are most likely to depend on the state of the ovary of the animal at the time of treatment. Animals with ovaries showing some follicular activity ("moderate anoestrus") may be successfully treated with a single dose of hCG and for animals in "deep anoestrus" hCG treatment must be preceded by priming with FSH or PMSG. The priming with PMSG or FSH is to initiate follicular activity prior to treatment with hCG, which has predominately LH-like activities. The recommended treatment protocol is given below

(a) Priming with PMSG (200-500IU/animal) or FSH (4-8 mg/animal) (one or two injections given 12-24 hours apart)
(b) Follow-up with HCG (1000-3000 IU/animal) given 12-24 hours after the last PMSG or FSH injection.

15.1.3.3 *Use of short-term progestagen therapy:* Progesterone has been tried successfully in inducing oestrus in anoestrous animals. The rationale behind the use of progesterone is that the exogenously administrated progesterone will cause the build up of LH reserves at the pituitary, so that upon withdrawal of exogenous progesterone, the pituitary will be able to release LH in large quantities, which will then initiate follicular development and hence ovulation ("rebounding effect").

Recommended treatment protocols are given below.

(a) *Short term progestagens (Progesterone - releasing intra-vaginal devices and progesterone - impregnated silastic implants):* Either of the above is left *in situ* for 9-10 days. Animals are expected to show oestrous signs with 2 - 4 days after withdrawal of the device. The

disadvantage of this therapy is that the induction rate as well as the conception rate are not satisfactory.

(b) *Short-term progestogen with FSH or PMSG*: Either PRID or a silastic implant is left inside for 9- 10 days and at the time of withdrawal of the device, 8-10 mg of FSH or 200-500 IU of PMSG is given. Animals are expected to show signs of oestrus within 2 - 4 days. Induction and conception rates following such treatment appear to be satisfactory.

In both situations, two inseminations at 48 and 72 hours or one insemination at 60 hours after the withdrawal of progestogen have been recommended. On-station and on-farm trials have shown that the success rate with this treatment to a large extent depends on the nutritional status of the animal. Animals that are adequately nourished and healthy would respond better than those that are in poor body conditions[1]. Similarly, the conception rates higher when the induction is done during favourable seasons of the year (e.g. rainy season).

Therefore, the recommended treatment plan for handling anoestrus in cows and buffaloes is as follows.

(a) If the body condition score of the animal is lower than 2, then the most important and effective remedy is to improve the quality of feed given to the animal so as to correct any imbalances in the feed (refer Chapters 1 to 6).

(b) If the cow is being still suckled, then reduce the intensity of suckling by the young by resorting to limited suckling (once or twice a day suckling) or removal of the inhibition completely by weaning the calf. It must be noted that cow-calf interaction (e.g. visual contact) too is as inhibitory as suckling *per se* and therefore, the calf must be kept in a place which would provide minimal visual contact between the mother and calf[2].

(c) If the above two approaches do not resolve the problem and provided that the body condition score of the cow is above 2+, then attempts could be made to stimulate ovarian function and hence the cyclicity by using exogenous hormones.

15.2 Control of oestrous cycle for timed insemination

The oestrous cycle of an animal to be bred could be controlled by pharmacological treatment. The advantages of such efforts are, (i) the alteration of the intrinsic reproductive rhythm of the animal according to wishes and requirement of man, (ii)

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1 Abeygunawardena *et al.* (1996a)
2 Abeygunawardena *et al.* (1996b)

saving of labour cost which would otherwise be spent on activities such as heat detection and meticulous record keeping and (iii) inseminating the animal at a predetermined time without reference to the state of behavioural oestrus. The latter could overcome the penalties associated with incorrectly timed inseminations, which result in fertilisation failures and hence low conception rates. The modifications of the oestrous cycle of cycling animals may include (i) control of oestrus for timed insemination of an individual cow and (ii) control of oestrus in a group of animals in synchrony for timed insemination (oestrous synchronisation).

As stated earlier, the oestrous cycle in all species of domestic animals is composed of hormonally controlled, well defined phases called luteal and follicular phases, the durations of which are fairly constant within a given species. This is particularly true as regard the follicular phase. For example, if the luteal phase of a cycling animal is terminated (e.g. by manual removal of CL or induce luteolysis by pharmacological substances), the follicular phase is initiated and the animal may return to oestrus in a predictable manner in few days. For example, it has been shown that the demise of the CL in cattle results in animals returning to oestrus in 3 to 4 days. Similarly, if the luteal phase is artificially extended beyond the normal length of the luteal phase, the animal will commence the follicular phase upon termination of the exogenous progesterone source, resulting in the animal returning to oestrus within 3 to 4 days. The latter treatment has additional beneficial effects in that the exogenous progesterone acts as an inhibitor to gonadotrophin release, thereby favouring the development of LH reserves at the pituitary, which are released in large quantities upon withdrawal of the progesterone source. The large quantity of LH released promotes follicular development and subsequent ovulation and hence the ability to catch animals in anoestrus too.

15.2.1 Termination of luteal phase

(i) *Anucleation of CL*: The earliest and most primitive approach has been the removal of the CL per-rectally (anucleation). However, this treatment is not favoured now because it would lead to haemorrhage and subsequent adhesion formation.

(ii) *Use of prostaglandins*: The more recent and currently popular method is the use of prostaglandin F2 as a luteolytic agent. Both naturally occurring and synthetic analogs have been widely used effectively.

The CL of many species is however sensitive to PGF_2 only during a short period of time. For example in the bovine, the CL is sensitive only between day 5 and day 15 of the cycle (mid-luteal stage). Therefore the stage of the oestrous cycle with regard to the luteal phase needs to be ascertained accurately. The need for such accurate determination of the stage of the oestrous cycle could be avoided, if one adopts a two-dose regime. In this approach the animal which is at mid-luteal phase will respond to the first injection and will return to oestrus 3 to 4 days post-PGF_2 injection. The animal could be served either during this oestrus or skip the oestrus and allow the animal to reach the mid-luteal phase. Whereas the non-responder which could have been served either at early luteal (0 to 5 days) or late luteal phase (15 to 20 days) will be in mid-luteal phase in 9 to 10 days after the first PGF_2 injection. The second

injection given 9 to 10 days later will catch both the responders and non-responders to the first PG injection and animals in both groups return to oestrus in 3 to 4 days after the second PGF_2 injection. Therefore, any cycling animal irrespective of the stage of the oestrous cycle to the first PG injection will become sensitive to the second prostaglandin injection if it is given 9-11 days after the first injection. The animal will be in oestrus in 3-4 days time and could be inseminated at a pre-determined time (e.g. usually two inseminations at 72 and 96 hours after the second injection or single insemination 80 hours post-injection). Clinical trials have shown that the 2 dose regime has given higher conception rates than the one-dose regime, because the success of the latter depends on the ability of the veterinarian to estimate the stage of the oestrous cycle.

15.2.2 Extension of luteal phase by exogenous progesterone

The most recent development has been the use of exogenous progesterone to extend the luteal phase. The earliest trials in which exogenous progesterone therapy was tried over 21 days, revealed that fertility was not very good, perhaps due to prolonged administration of progesterone. Consequently, trials were performed with short lengths (9 to 11 days) of progesterone therapy. But when this approach was used, the treatment protocol was not able to catch animals that are either in early luteal phase (day 0 to 5 of the cycle) or in late follicular phase (day 18- 20 of the cycle). But this problem has been overcome by inclusion of proestrogen and oestrogen as bolus injections at the time of commencement of progestagen therapy. Oestrogen prevents or inhibits the formation of the CL from a recently ovulated follicle, while progesterone prevents pre-ovulatory follicular maturation and ovulation.

Hence, progesterone now is used in combination with oestrogen to control the oestrous cycle in cycling animals for timed breeding. There are several preparations in the market now. Progesterone releasing intra-vaginal device (PRID) and Progesterone sub-cutaneous implants (e.g. Crestor) have been used successfully for timed breeding. With both preparations, the protocol involves leaving the device *in situ* (PRID in the vagina or Crestar under the skin) for 9-11 days and animal returns to oestrus in 2-3 days after the withdrawal of the device. Two timed insemination at 48 and 72 hours after withdrawal have given acceptable levels of fertility. The success rate has been further improved by combining the progesterone therapy with GnRH or PMSG given at the time of implant or PRID withdrawal.

Treatment regimes for controlling oestrous cycle for timed insemination in cattle and buffaloes are:

(a) *$PGF_{2\alpha}$ and its analogs*

(i) Single dose regime to animal in the mid-luteal phase and insemination at 72 and 96 hours after treatment or a single insemination at 80 hours after the injection.

(ii) Double dose regime (day 0 and day 9 to 11) to cycling animals, irrespective of the stage of oestrous cycle and two insemination

at 72 and 96 hours after the second injection of PG or single insemination at 80 hours.

(b) *Progesterone releasing devices*

(i) Progesterone-releasing Intra-vaginal Device (PRID) - the coil is inserted into the vagina and left *in situ* for 9-11 days. Double insemination at 48 and 72 hours after the withdrawal of the coil or a single insemination at 60 hours.

(ii) Progesterone releasing subcutaneous silastic implants sub-cutaneous implant is left *in situ* for 9-11 days. Double insemination at 48 and 72 hours after the withdrawal of implant or single insemination at 60 hours.

15.3 Control of ovulation

It was established elsewhere, that the time of ovulation during oestrus is regulated by a surge release of gonadotrophin hormone (i.e. LH) which is provoked by the positive feedback action of oestrogens. It was also mentioned that if the onset of behavioural oestrus could be detected with precision, it follows that the time of ovulation could be estimated with accuracy. In some instances, however, despite mating at optimum time following the onset of the oestrus, conception fails to occur resulting in repeat breeding. It is well known that there are a wide variety of causes of repeat breeding and one of the possible causes is the lack of synchrony of ovulation with onset of behavioural oestrus. As a treatment approach, one could attempt to induce ovulation with exogenous gonadotrophin and animals could then be bred at a pre-determined time. This could be achieved with a hCG or GnRH bolus injection of hCG (2000 IU) or GnRH (250 μg) at the beginning of behavioural oestrus, which will promote the final maturation of the developing follicle. If the exogenous gonadotrophin is given too early, it may result in the shedding of the primary oozyte rather than the secondary oozyte. Administering the exogenous hormone too late may not be advantageous as the endogenous LH surge would have already occurred. Ovulation could occur within short period of time as opposed to variable and protracted period under natural conditions. Service or insemination 18 to 24 hours after administration of exogenous hormone in cattle, 18-24 hours after in sheep and 24-36 hours in pigs have been recommended to achieve good results..

15.4 Embryo transfer

Embryo transfer refers to the technique by which fertilised oocytes or embryos are collected from a female (donor) and transferred for development to term in another female (recipient).This technique has provided an opportunity to multiply the value of a superior female, as in the case of artificial insemination in the male. Under normal farming practices, a cow could produce only about 4-10 progenies, even though, the ovaries of these cows contain thousands of primordial follicles which are capable of developing into viable oocytes, and if the opportunity is given, into viable progenies.

Application of embryo transfer technology will help:

(i) To increase the number of offspring that could be obtained from a genetically superior cow.
(ii) To transport genetic material across the county and across countries. This method offers an advantage over the transport of live animals which is costly and involves the risk of spreading diseases.
(iii) To perform sexing in order to increase the number of female offspring born from a superior cow.

15.4.1 Preparation of donor animal

A cow with genetically superior characteristics for productivity and other desirable characteristics could be induced to produce more than one follicle destined for ovulation during the ensuing oestrus. This technique is called super-ovulation. This could be achieved by administration of gonadotrophin. One of the recommended protocols for this superovulatory treatment is given below (Fig. 15.1).

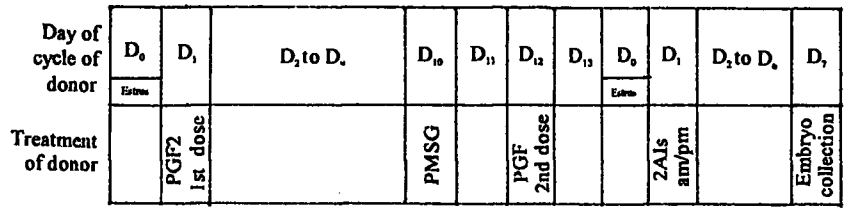


Fig. 15.1 Super-ovulatory treatment protocol for donor cow in an embryo transfer programme

A cycling cow is treated with a suitable preparation (Prostaglandin or progestogen) to induce oestrus on a pre-determined date. Trials have shown that the highest success rate in inducing the development of normal ovulatory follicles could only be achieved during the mid-luteal phase. The early phase would produce a lower number of follicles, while the late luteal phase results in development of cystic follicles. On day 12, the corpus luteum of the induced cycle is made to regress by giving prostaglandin. Animal will be in oestrus by day 14 (2 days after PG injection) and insemination is done on days 15 and 16 (minimum of 3 inseminations at 8 hour intervals with higher concentration of sperm than used for routine inseminations)

15.4.2 Preparation of recipient animals

The recipient animals involved are prepared for synchronisation of the stage of the oestrous cycle of the recipients with that of the donor. This could be achieved with a 2 dose - $PGF_{2\alpha}$- injection regime, as shown below (Fig. 15.2).

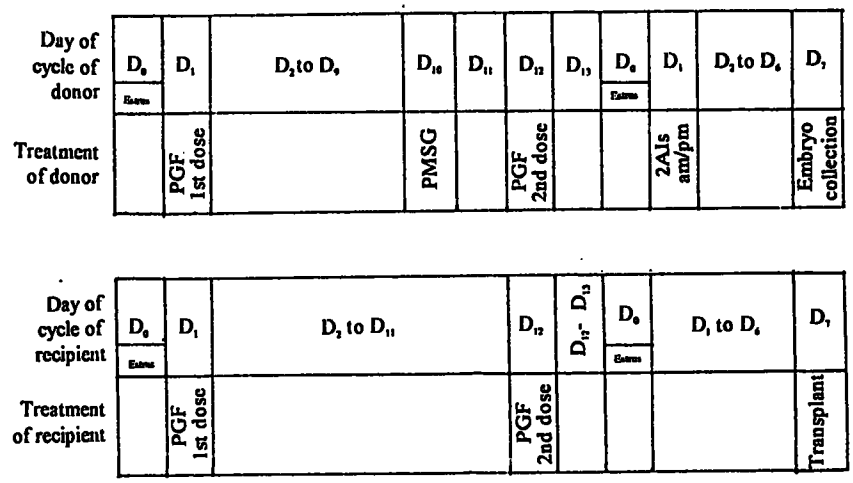


Fig. 15.2 Synchronisation schedule to prepare recipients in an embryo transfer programme.

As shown above, the protocol of $PGF_{2\alpha}$ - injections depends on the protocol of superovulatory treatment.

15.4.3 Insemination of donors

Insemination of the donor animal is usually done at a pre-determined time using a higher dose of semen than for normal insemination. Several inseminations are performed at short intervals of time (3 inseminations in 8 hour intervals has been recommended).

15.4.4 Collection of embryos

Collection of embryos has been attempted by either of two methods (a) non surgically or (b) surgically.

In cattle, embryos enter the uterus by 72 to 96 hours after fertilisation. Usually the collection is attempted between 5 to 12 days post insemination. This correspond to the morula through blastocyst stages in which the conceptus is still covered by the zona pellucida and appears to be less prone to damage while handling.

(I) Surgical collection - This involves the opening of the animal's body cavity and reaching the uterus through a body wall incision. The uterine horns are grasped and both ends are ligated. The uterine horn contents are flushed out using a suitable medium (e.g. M199 or phosphate buffered saline) as shown below, using a syringe, blunt needle and a catheter, into a vessel containing suitable culture medium (Fig. 15.3).

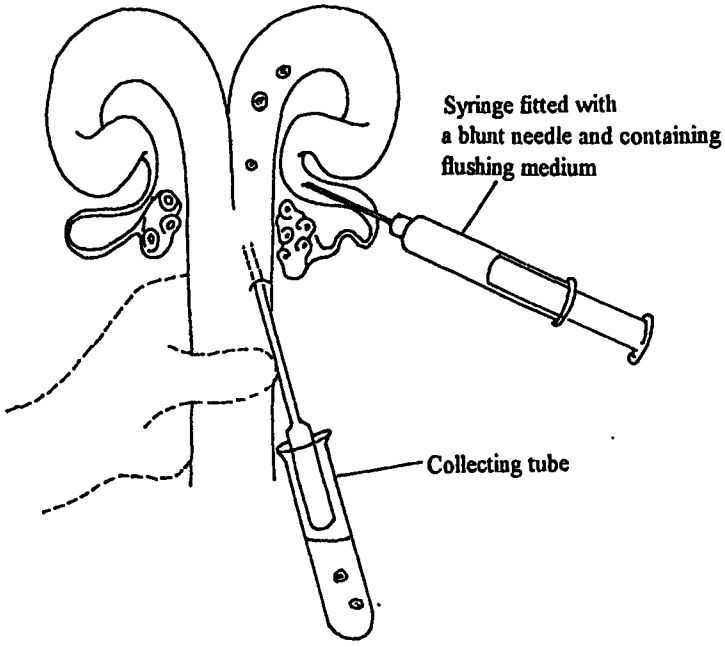


Fig. 15.3. Diagrammatic presentation of surgical technique of recovery of embryos from a super-ovulated cow.

This method usually provides higher recovery rates than non surgical collection. But it has its own disadvantages. This technique requires the services of a well trained team (i.e. surgeon, anaesthetist) and the animal become less fertile at subsequent oestruses, because of the possibility of adhesion and scar tissue formation.

(ii) Non surgical collection - This involves collection of embryos through the cervix and vagina. This is the most accepted technique but gives lower recovery rates than surgical collection. It has several advantages over the surgical collection. Further this could be carried out with less number of people and even with semi-skilled personnel.

This procedure involves the passing of a catheter (i.e. 3- way or 2-way catheter) through the cervix into the uterine horns and flushing the contents with a suitable medium to recover the embryos (Fig. 15.4).

15.4.5 *Evaluation and storage of embryos:* Embryos recovered from the superovulated cow into flushing medium is evaluated for viability before direct transfer or storage. A preliminary assessment of embryonic viability and also differentiation between embryos and unfertilised oocytes can be made by examination under the dissecting microscope. Healthy, viable embryos could be identified from the characteristic presence of blastomeres of uniform size, number of cells and the shape

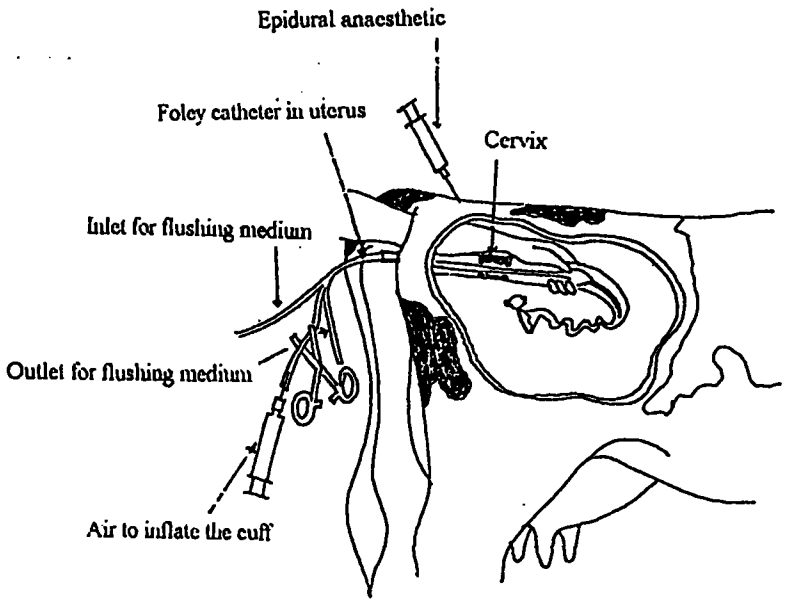


Fig. 15.4 Diagrammatic presentation of non-surgical technique of recovery of embryos from a super-ovulated cow.

of conceptus which correspond to its age. For example, 4 to 5 days old embryos consist of 16 cells; 5-6 days old embryos consists of 32-64 cells, 7-9 days old embryo must be in the blastocyst stage, etc.

The freshly recovered embryos could be kept at room temperature or refrigerated temperature for 6- 12 hours. Embryos could be kept viable for a longer period under deep frozen conditions in liquid nitrogen. Freezing, however requires very precise conditions such as reducing the temperature to -196°C at a controlled rate, unlike in freezing semen. Further, when deep frozen embryos are prepared for transfer, the thawing process to bring the temperature to body temperature level must also done at a controlled rate. Both processes (freezing and thawing done with the aid of computer controlled devices), are to be done with absolute precision. Embryos in a deep frozen state can be kept for an indefinite time period but the pregnancy rate following freezing and thawing is somewhat reduced (40-50% reduction).

15.4.6 *Transfer of embryos:* Transfer of embryos is usually attempted non-surgically, through the cervix. For successful embryo transfer, synchrony between the stage of embryos and reproductive tract of recipient is also absolutely essential. To achieve optimum pregnancy rates following transfer of embryos, the donors and recipient oestruses must be closely synchronized within a variation of ±12 hours.

15.5 *In- vitro* fertilisation of oocytes

Attempts are underway to master the techniques of *in-vitro* maturation of entire follicles or oocytes. Following maturation, the oocyte is fertilised with capacitated sperm. The embryo is cultured in the laboratory before storing for future use. Much

progress has been made in this regard, but a great deal of work remains to be done before the technique could become a commercial possibility.

These techniques offers greater advantages over multiple ovulation and embryo transfer as they offer the possibility of the use of ovaries which would otherwise be discarded at slaughter.

Selected references

Abeygunawardena, H., Kuruwita, Y.V. and Perera, B.M.A.O. (1996a). Effects of different suckling regimes on postpartum fertility of buffalo cows and growth and mortality of buffalo calves. *The Role of the Buffalo in Rural Development in Asia*, SAREC/NARESA Press. pp. 321-336.

Abeygunawardena, H., Kuruwita, V.Y. and Perera, B.M.A.O. (1996b). Effects of exogenous hormones on fertility of postpartum anoestrous buffaloes. *The Role of the Buffalo in Rural Development in Asia*, SAREC/NARESA Press. pp. 337-350.

Peters, A.R. and Ball, P.J.H. (1995) *Reproduction in Cattle.* (2nd edition). Blackwell Science Ltd. Oxford, UK.

Hafez, E.S.E. (1987) *Reproduction in Farm Animals.* (6th edition). Lea and Febiger, Philadelphia, USA.

CHAPTER 16

Scientific Basis for Genetic Improvement of Buffaloes and Cattle for Milk Production in Sri Lanka.

M.G. Jeyaruban , H. Abeygunawardena and A.D.N. Chandrasiri

Introduction

Buffaloes and cattle indigenous to Sri Lanka are small in body size and poor producers of milk but fertile even under hostile environments. This is the outcome of natural selection over centuries of these animals for survival without intervention by man. High fertility even under adverse environmental conditions is the key to success in the traditional, extensive production systems characterised by grazing on communal grazing land prevalent in many areas of dry and intermediate zones of the country. Unfortunately, the traditional system has to changed with the demographic and socio-economic changes taking place in the country. Since independence, the need for a productive livestock sector has become more important. One of the strategies adopted in this context is the genetic upgrading of indigenous cattle and buffalo population.

As early as the 1950s, there had been in Sri Lanka, a fairly clear concept of the appropriate dairy cattle breeding policy that was suitable at that time, thanks to the pioneering work done by early researchers. Breeding trials performed at Karangoda Uyangoda indicated that even under optimum conditions of selection, the rate of genetic improvement in milk yield in the base population would hardly exceed 10% of the average yield per year. Obviously this does not hold out much hope of rapid improvement in a herd (indigenous zebu cattle and buffaloes) with an average lactation yield of under a hundred gallons (approximately 500 litres). Alternative breeding systems, therefore have been tried out, such as crossing the local stock with exotic breeds with superior performance potential and interbreeding the progeny with a view to evolving a new type. The results of crossbreeding experiments which were conducted at Karagoda-Uyangoda, Undugoda and Weerawila livestock farms led to the following conclusions, that (1) milk production of F_1 European x Zebu crosses was markedly superior to that of indigenous the Zebu base population; (2) interbreeding of the F_1 progeny resulted in a marked decline in milk yield by 30-40% at F_2 level; (3) backcrossing the F_1 to European breeds resulted in a progeny population whose performance levels vary considerably with variation in climatic and management conditions and (4) performance of F_1 was not only influenced by the genetic merit of the European parent but also by the genetic merit of the local Zebu. According to these findings, Sri Lanka was left with the option of going either for new breed formation as was carried out in Jamaica (Jamaica Hope), Australia (Australian Milking Zebu-AMZ and Australian Friesian Sahiwal-AFS) and many other countries, or the adoption of cris-crossing or rotational cross-breeding systems.

The breeding programmes conducted both by the Government and private sector, thus far has been directed at producing animals with high production potential compatible with a given agroclimatic zone of the country. The national breeding policy and the guidelines for field staff have been issued time to time by the agencies responsible for implementing the genetic upgrading programmes of the country (refer Chapter 17 for details).

16.1 Theoretical basis of crossbreeding of farm animals

Crossbreeding strategies that are implemented in Sri Lanka can be classified into, (1) breed replacement strategies (grading up of the existing stock to desired exotic blood levels) and (2) establishment of stable crossbreeding systems and formation of synthetic populations.

Benefits from crossbreeding arise due to heterosis effects between crosses. Heterosis is the deviation between the cross and mid-parent mean. For example, if the average milk yield for 305 days of lactation of the local, Jersey and local x Jersey crosses are 2000, 4000 and 3200 litres, respectively, the heterosis for milk production between local breed and Jersey is

$$= [(3200 - ((2000 + 4000) / 2) / (2000+4000)/2] * 100 = 200/3000 * 100 = 6.67\%$$

16.1.1 Breed replacement strategies (grading up to exotic level)

The breed replacement strategy is aimed at bringing about a gradual change in the genetic composition of a population. Theoretically, more than 90% of genotype of the local population could be replaced within four generations of cross breeding. Selected bulls or semen of a chosen genotype could be used on the local base population. For example, semen from *Bos taurus* bulls is used to upgrade the existing cattle population in most of the wet zone areas (e.g. Hill and Mid country regions) while bulls of exotic *Bos indicus* are used to upgrade the local cattle and buffaloes in the dry zone. The breeding chart is given in Fig. 16.1, where E stands for exotic breeds, L for the local breed and C for the crossbred progeny.

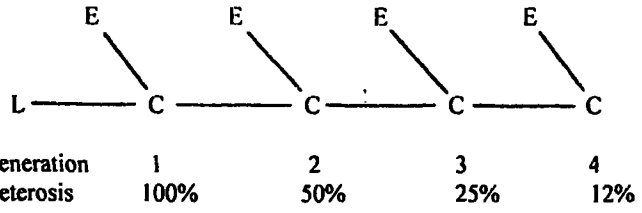


Fig. 16.1 Breeding chart for upgrading local genotype up to the exotic blood levels.

As shown in Fig. 16.1, after the first two to three generations, the amount of heterosis contributing to performance become negligible. The advantage of upgrading is that although a considerable period of time is required before the local population is replaced, it causes the least disruption to local husbandry practices, as farmers have ample time to adjust their husbandry practices to suit new emerging genotypes.

16.1.2 Formation of synthetic population

The aim of formation of a synthetic population is to combine the high production potential of *Bos taurine* breeds with the adaptability of local breeds. Among the *Bos taurine* dairy breeds, Friesian and Jersey appear to have the best ability to combine with other breeds. The levels of exotic blood stabilized in the synthetic population depend on the objective of the cross breeding programme and on agro-ecological conditions and the resource base. The most commonly practised crossbreeding strategies are stabilization at 50% (Fig. 16.2) and stabilisation at 75% (Fig. 16.3) exotic blood levels.

16.1.2.1 *Stabilisation at 50 exotic blood level:* Maintaining the herd at 50% exotic blood level can be done by *two ways.* The first method is by *inter se* crossing of 50% Temperate X Zebu bulls with 50% Temperate x Zebu cows (Fig. 16.2a). But due to breakdown of non-additive genetic interaction, some of the F_2 cows might produce lower milk yields than the F_1 cows. This could be avoided (up to a certain extent) by crossing with another distinctly different synthetic dairy breeds (e.g. 50% Friesian x Local and 50%Jersey x Local). The other method is the use of F_1 bulls by crossing the local selected female (L_1) with desired exotic (E) bulls and then using them on the local population. (L). The resulting cross (C_1) is mated to another F_1 from a suitable breed combination. In this method as shown in 16.2(b), where 50% exotic blood level is achieved only after the 4th generation. This has the added advantage of retaining a considerable degree of heterosis.

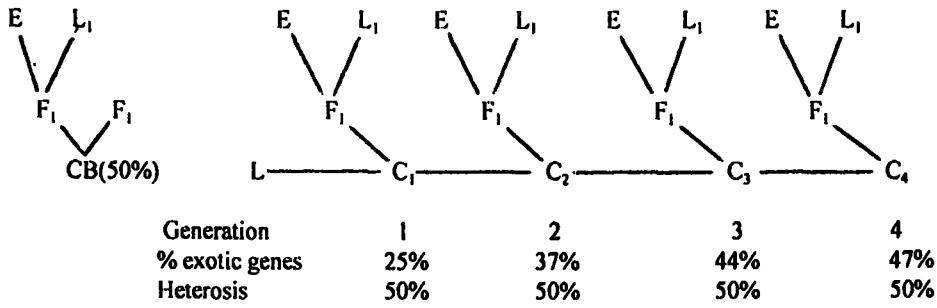


Fig 16.2(a) **Fig 16.2(b)**

Fig. 16.2 Stabilization at 50% exotic level.

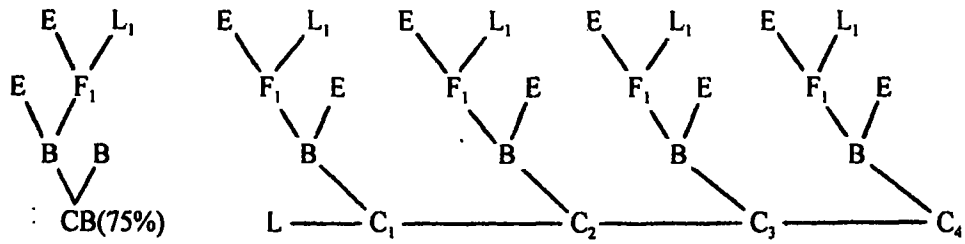
16.1.2.2 *Stabilisation at 75% exotic blood level:* If a higher degree of exotic genes is required, but still short of 100%, grading up to 75% can be practised. This can be done by *interse* mating of three-quarter bred males to the females of the same genetic constitution (Fig. 16.3a) or continuous use of three-quarter bred males on local cow population (Fig.16.3b). In the second method, the exotic bulls (E) are mated to selected females in the local population (L_1) to produce the F_1 generation, which is backcrossed to the exotic males to produce 75% exotic males (B). These males are then used on the local population (L). The maximum heterosis of 75% in the first generation will gradually drop to 37.5% over successive generations (16.3b). In this method as shown in 16.3(b), where 70% exotic blood level is achieved only after the 4th generation.

It must be stressed here that both breed replacement and formation of synthetic populations require selection to be practised simultaneously, if the desired results are to be achieved.

16.2 Theoretical basis of selective breeding

Selective breeding of livestock is the method of improving the genetic quality of a very heterogenous population. The basic steps are (1) identification of traits which are important to the production system (eg. milk yield, fat percentage, birth weight, growth rates, age at first puberty, etc.), (2) identification of animals which are of superior genetic merit for traits of interest and (3) use of animals with the highest performances with regard to the chosen characteristics, as parents of the next generation.

It must be emphasized here, that the response to any selection from a population depends on three criteria; that is the (i) heritability of the chosen trait, (ii) selection intensity and (iii) heterogeneity of the base population for the chosen trait.



Generations	1	2	3	4
% exotic genes	37%	56%	65%	70%
Heterosis	75%	Slight decrease in successive generations		

Fig. 16.3(a) **Fig. 16.3(b)**

Fig. 16.3 Stabilization at 75% exotic level.

16.2.1 Heritability:

Heritability (h^2) is defined as the ratio of the additive genetic variance (V_A)to the phenotypic variance (V_P), where $h^2 = V_A/V_P$. In general terms, this refers to the proportion of the total phenotypic variation that is caused by genetic differences between members of a population. If a character is heavily influenced by genetic factors and the influence of the environment is low, the heritability of such a trait is high. Conversely, if a character is heavily influenced by non-genetic or environmental factors, then the heritability of such a trait is low. Selection for traits with high heritability is easy and the animals' own records (mass selection) could be applied. But progeny testing or pedigree analysis is required for the selection of traits with low heritabilities . The estimated heritability values for some of important traits are summarized in Table 16.1.

16.2.2 Selection Intensity

Selection intensity refers to the proportion of the population selected as parents of the future generation. It is well known that any population with respect to a particular trait is normally distributed which could be characterised by the population mean and the standard deviation. If the average milk production of a population is 2000 litres over a 305 day lactation, and the average production of the selected population (top 30% of cows) is estimated to be 3500 litres over a 305 day lactation, then if the best animals are selected from the existing population as the parents of the next population, the progeny will have a higher expected average than the base population. Continuation of this process in successive generations will result in increasing the mean value of the trait under consideration from generation to generation. The higher the selection intensity (e.g. 30% vs 50% included as parents of the future generation), the lower will be the proportion selected for breeding. Higher selection intensities are generally applied for traits of economic importance and mass selection is practised in these situations.

Table 16.1 Heretability estimates for important traits of cattle and buffaloes

Trait	Estimate	Action	Outcome
Calving interval	0 - 15	Give more emphasis to records of dam and relatives during selection	Takes more generations for improvement
Milk yield	25 - 40	Use relatives' and cow's own records in selection	Moderate response to selection
Fat yield	27 - 43	Use relatives' and cow's own records in selection	Moderate response to selection
Fat %	32 - 87	More emphasis for cow's records	Faster genetic progress
SNF	53 - 83	More emphasis for cow's records	Faster genetic progress
Protein %	48 - 88	More emphasis for cow's records	Faster genetic progress
Physical appearance	30 - 60	More emphasis for cow's records	Faster genetic progress

In this process, if the variation within a population for the chosen trait is high (as shown in Fig. 16.5), this will result in higher response to selection in the progeny population.

16.2.2.1 *Heterogeneity of the population*: The extent of genetic variation present in the population will have a strong bearing on the response to selection. As stated earlier and shown in Fig 16.4, any population with respect to a particular trait is normally distributed with a population mean and the variation.

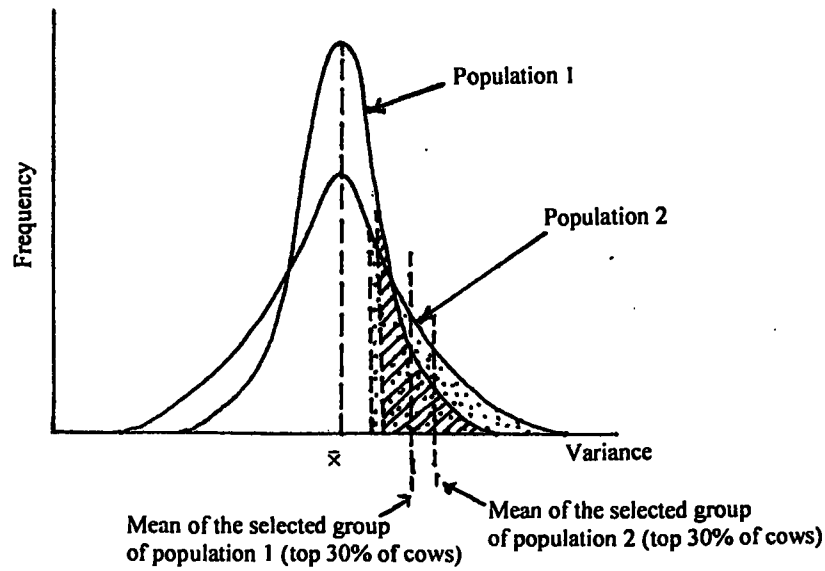


Fig. 16.4 Amount of genetic variation and response to selection

In Fig 16.4, population 1 is more uniform in genotype for the trait of interest than in population 2, as the animals being all closer to the mean. Thus the genetic variation for a chosen trait in population 1 is lower than that of population 2. Selection of the top 30% of animals from each population produces selected parent groups with different means. Due to the higher genetic variation in population 2, the mean of the selected parent group is higher. Therefore, rate of genetic progress through selection will be greater in population 2 than in population 1.

As described above, the response to selective breeding could be optimized by (a) selecting for traits with high heritabilities, (b) using high selection intensities and (c) selecting from a large population and (d) pooling information from many sources. With regard to dairy cattle breeding, in practice, cows are selected on the basis of the first lactation performance, as the heritability estimates obtained from the first lactation are consistently higher than those from subsequent lactations. In the case of bulls, the performances of daughters are used as criteria for selection.

16.3 Selection at the farm level

Improvement programmes in many countries have been based on selection of the best animals within a herd as parents of next generation. Cows may be selected on one or more of the following three criteria. (1) individual records of cows. (2) the pedigree records of the parents and (3) phenotypic appearance of breed specific desirable traits. Of these three, records of production performance offer the most reliable means for selecting cows for breeding. The following traits could be considered in the selection programmes: (1) age at first calving (2) first lactation yield (3) first lactation length (4) 305 day lactation yield (5) days between first calving and conception and (6) physical appearance. However, one must note that as shown in Table 16.1, the response to selection is faster and remarkable when selection is applied on the basis of traits which have high heritabilities.

16.4 Performance recording and selection

Reliable records are absolutely essential for implementing a suitable genetic upgrading programme. Records will help to (1) remove animals from breeding herds based on age and productive and reproductive performance (2) select animal as replacement stock of the herd and (3) evaluate the performance levels of the herd, including production, reproduction and economic indicators. Recording and monitoring must be implemented at two levels, i.e. at farm level and national level

16.4.1. Recording at farm level

Farm records should include the following.

(a) date of birth of the cow / bull
(b) dam's number
(c) sire's number (or semen code for AI born animals)
(d) date of first service and subsequent services
(e) service bull number / semen code number
(f) date of calving - with calf's weight and sex
(g) daily/monthly and lactation milk yield

(h) lactation length
(i) dates and details of veterinary interventions

In implementing the national breeding programme farms that maintain accurate records could be registered as nucleus farms and these could be recommended for issue of breeding animals to other farmers. The registered farms could be provided with imported type I semen or proven bulls at low cost. A higher purchase price could be offered for proven daughters and also free advice on feeding and management as an incentive.

16.4.2 Recording at national level.

Recording at national level should be the responsibility of Extension Officers from the Department of Animal Production and Health or by approved officers of farmer societies. Recording could be done at monthly intervals on each livestock owning household. The recording could include the date of breeding and calvings, identity of bulls used, body weight, body condition score, milk yields, dates and details of veterinary interventions and disposals and purchases and slaughter of stock. Such recording and monitoring systems (e.g. milk recording system) coupled with AI service will help to identify the super dams in the national herd. Data from the national level recording system could be computerized and these could be used to select the most outstanding cows as bull mothers.

Bull calves from such superior bull mothers can be used in the national breeding programme - as semen donors or stud bulls for natural breeding programmes. The data drawn from the farmer level recordings could be used to rank cows within the herd to evaluate the production capabilities of animals. Breeding value of cows for different traits could be derived as deviations from the herd average.

Selected references

Cunningham, E.P and Syrstad, O (1987) *Crossbreeding* Bos indicus *and* Bos taurus *for milk production in the tropics.* FAO Animal Production and Health Paper No. 68.

Dalton, D. C. (1985) *An Introduction to Practical Animal Breeding.* Collins, Grafton Street, London W1.

Falconer, D. S. (1981) *Introduction to quantitative genetic* (2nd edition) Longman, London & New York W.H. Freeman and Company, San Francisco, USA.

Schmidt, G.H. and Van Vleck, L.D. (1973) *Principles of Dairy Science.* W.H. Freeman and Company, San Francisco, USA.

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CHAPTER 17

Breeding of Buffaloes and Cattle

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Introduction

The indigenous buffalo (Lanka buffalo) and zebu cattle ("batu harak") of Sri Lanka are well adapted to tropical, often harsh climatic conditions and are resistant to many tropical diseases. But they are small in body size and low in milk production compared to their counterparts in the Indian subcontinent and in temperate countries. However, they are highly fertile, provided they are well fed, and they reproduce efficiently even under harsh climatic conditions. The relatively small body size and low milk production has been the result of inbreeding in closed herds over several centuries and the absence of selection for production traits by man. These animals are predominantly found in the dry and intermediate zones of the country. In the wet zone, however, indigenous cattle have been replaced gradually by more European dairy type animals ("cape" cows). Some cross breeding of buffaloes with improved dairy types from the Indian sub-continent has also taken place but on a very limited scale.

Genetic upgrading of cattle and buffaloes commenced as far back as the 1950's, both through natural breeding and artificial insemination, with a view to improve the productivity of the dairy industry. Unfortunately, there has not been rigorous monitoring and selection of the upgraded populations and most often both the extension worker as well as the farmer have failed to adhere to the breeding recommendations issued by the line ministries and related agencies. As a result, the desired benefits of 50 years of upgrading efforts have fallen short of expectations. For example, in the wet zone areas, the productivity of European type) cows has gradually declined and it is conceivable that this is a result of inappropriate breeding practices and nutritional inadequacies in feeding systems.

17.1 Methods of breed improvement

Cattle: Genetic potential of Indigenous as well as crossbred cattle can be improved by crossbreeding with imported tropical breeds (from India, Pakistan and Australia) or temperate breeds (from Europe, America and Canada).

Buffaloes: As a method of improving the genetic potential for milk production of indigenous buffaloes, crossbreeding with imported river type buffaloes (from India and Pakistan) is feasible through natural breeding and/or artificial insemination (AI).

17.2 Importance of selecting the correct type of bull or semen

The selection of the correct type of bull or semen for breeding cows in smallholdings is the most important aspect in the breed improvement programme. The breed type must be able to withstand the climatic and management conditions prevailing in the area. This is because the calf that is produced through the upgrading programmes must

be able to survive, grow, produce and reproduce under the climatic conditions and on available feed resources of the particular area. Thus the artificial insemination technician and the farmer must be aware of the principles used in the selection of a bull or semen.

This chapter outlines the recommendations made by the National Breeding Committee, which consists of experts in the fields of animal genetics and breeding, for breeding of buffaloes and cattle in the different agro-climatic zones of Sri Lanka. The veterinarian and the artificial insemination technician must appreciate the importance of educating the farmer to enable him to select the correct type of semen for his animals. Participation of all three groups (veterinarians, AI technicians and farmers) in this process is extremely important.

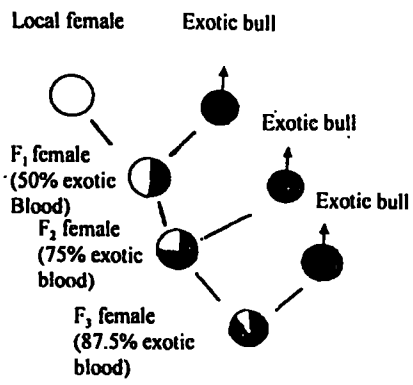
17.3 Different types of upgrading programmes

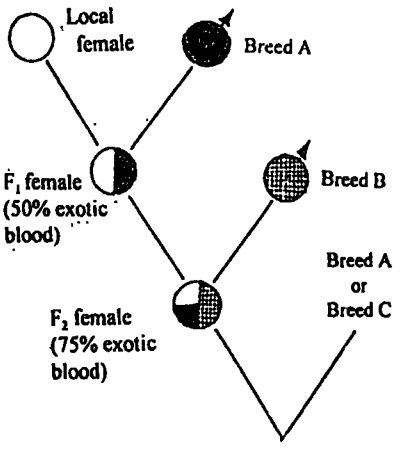
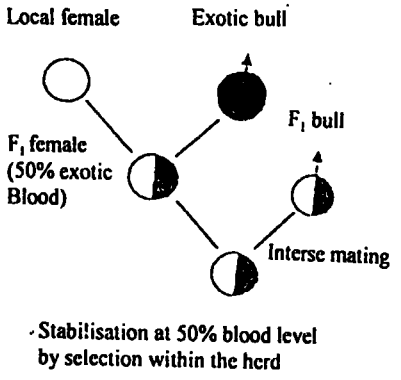
Genetic upgrading of animals could be achieved through several approaches. Three approaches adopted in Sri Lanka are (a) continuous upgrading, (b) rotational cross-breeding and (c) stabilising a desirable genetic composition by *inter se* mating. These three approaches are used in different regions of the country to develop suitable genotypes, which have the ability to thrive, produce and reproduce efficiently, as shown in Box 17.1.

17.4 Breeding of buffaloes

In order to upgrade the production potential of Lanka buffaloes, river type breeds such as Murrah, Surti and Nili-Ravi have been imported from India and Pakistan. They have been bred in their native countries for higher milk yield and also have the ability

Box 17.1 Different types of cross-breeding approaches

Continuous upgrading  	Cows of low producing breeds are mated to bulls of breeds with high production potential. The female progeny of subsequent generations continue to be bred to bulls of the same breed, thus gradually upgrading the production potential of the progeny to that of the breed of the bull.

Rotational cross breeding  	Here, the upgrading of the production potential of low producing animals is achieved by breeding successive generations alternatively to two or three superior breeds with high production potential, in rotation.
Stabilising desired blood levels by *inter se* mating  	In this system, low producing cows are bred to a bull with a higher breeding potential either to achieve a 50% blood level or higher (75%, 87.5% ... etc) in the first round of breeding. Thereafter *inter se* mating of desired generation (50% or above) is carried out to stabilise the blood level at pre-determined level (50% or higher level).

to perform well under tropical conditions. The characteristics of these breeds, their genetic potential for milk production and the location of the farms where they are found in Sri Lanka are given below (Plates 1 to 3).

Crossbreeding of Lanka buffaloes and exotic river type buffaloes produce healthy calves which have the potential to produce more milk than the local cow but less than the pure exotic buffalo. But by continuous crossbreeding with genetically superior pure bred males, the productivity of successive generations will gradually improve and ultimately reach levels as high as that of pure bred animals, provided they are managed and fed well.

17.4.1 Breeding recommendations for buffaloes

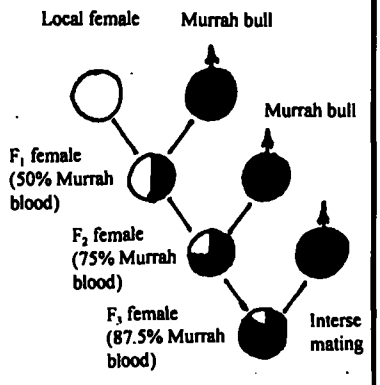
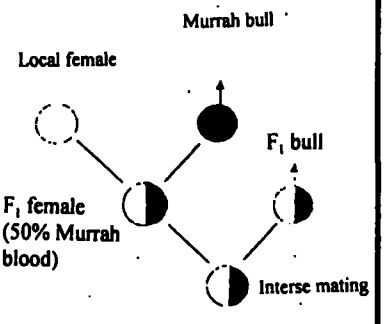
In all three major regions of Sri Lanka (wet, dry and intermediate zones), cross-breeding of indigenous (Lanka) buffaloes for continuous upgrading to the exotic river

Murrah buffalo  Plate 1	Murrah animals have been bred for high milk yield. They are large, with jet black skin and tightly curved horns. They can survive, reproduce and produce under hot and humid conditions. They have the potential to produce 3000-5000 kg of milk per lactation (10-16 litres/day), provided they are adequately fed. They are maintained at the NLDB Farms in Polonnaruwa and Ridiyagama.
Surti buffalo  Plate 2	Surti animals have been bred for high milk yield and butter fat content. They are smaller in body size than Murrah, their horns are flat, sickle shaped and directed downwards. The skin is greyish and the hair is grey to brown. They resemble Lanka buffaloes, but are better milk producers and are docile. They have the potential to produce 2000-4000 kg of milk per lactation (7-12 litres/day), provided they are adequately fed. They are maintained at the NLDB Farm at Melsiripura.
Nili-Ravi buffalo  Plate 3	Nili-Ravi animals have been bred for high milk yield and butter fat content. They are large, stocky animals with black skin and white markings on forehead, face, muzzle, legs and tail switch. Horns are short and broad at the base and closely curled back. They were introduced to Sri Lanka only recently, they have the potential to produce 3000-6000 kg of milk per lactation (10-20 litres/day). They are found at the NLDB Farm at Nikaweratiya and Mahaweli Farms at Kalankuttiya and Girandurukotte.

type has been recommended. However, the up-graded progeny need to be managed and fed well if their full genetic potential is to be realised. Therefore, two types of approaches are recommended, one for the intensive system and the other for extensive system of management (Box 17.2).

As shown in the diagram below, for intensive management systems, in the wet and dry intermediate zone and some areas of the wet zone, continuous upgrading of indigenous animals to 100% of exotic blood levels by back-crossing to the exotic river type pure-bred males is recommended. The offspring will be gradually transformed, through 50% exotic type in the first generation (F_1), 75% in the second generation (F_2), 87.5% in the third generation (F_3) and almost up to pure breed level after five generations. If the upgrading process continues by back-crossing to pure exotic buffalo bulls, an animal with production levels as high as those of exotic pure-bred animals could be produced with high adaptability to the local environmental and management conditions.

Box 17.2 Breeding recommendations for buffaloes

For intensive system  	The existing buffaloes must be upgraded using Murrah or Nili Ravi buffalo bulls, with continuous selection at each generation. As shown in the diagram, after nearly 5 generations (approximately after 15 years), the progeny would have more than 90% of genetic material derived from the river type bulls. Hence, there would be an incremental increase in milk yield from indigenous parent populations through F_1, F_2, F_3, F_4 and F_5, where the F_5 progeny is capable of producing milk at levels equivalent to those of exotic parent.
For extensive system -  	As the climatic and management conditions may not favour rearing 100% river type buffaloes, it is recommended that the existing population is upgraded to 50% exotic blood levels by cross breeding with Murrah or Nili Ravi buffalo bulls to obtain F_1 population. The F_1 population is mated among themselves (F_1 x F_1 - *inter se* mating) to stabilise the 50% blood level. Only the selected males of F_1 and selected females of F_1 must be mated to obtained the desired results.

But for the extensive system practised in most areas of the dry zone, where improvement of feeding and management practices would not be possible in the foreseeable future, upgrading of indigenous buffalo or existing crosses up to 50% exotic levels and stabilising the blood level by *interse* mating is recommended.

17.5 Breeding of cattle

As a result of the introduction of temperate dairy breeds during the colonial era and the availability of widespread AI services, the cattle population found in the country is very diverse and heterogeneous. In general, indigenous zebu animals and "up graded" zebus are primarily found in the dry and dry-intermediate zones, while more temperate dairy types, pure and "up graded" cows are found in the wet and wet-intermediate zones. Within these regions too, animals may exhibit variations in genotype. Further, this process of distribution of animals across the country has occurred primarily on the basis of the adaptability of breed types to agro-ecological conditions. For example, a temperate dairy type cow may not be able to produce and reproduce satisfactorily or even survive under harsh climatic conditions prevailing in the dry and dry-intermediate zones. Similarly, rearing indigenous or "up graded" zebu animals may not be economical in the wet and wet-intermediate zones, as the climatic conditions are more conducive to the rearing of better dairy type animals.

17.5.1 The recommended cattle breeds for genetic upgrading programmes

The Department of Animal Production and Health now recommends 4 breed types for genetic up-grading programmes across the country. The characteristics of these breeds, production potentials, suitability to climatic conditions and where the pure breeds are found in Sri Lanka are given below in Plates 4 to 7.

17.5.2 Breeding recommendations for cattle

Taking into consideration the variation in agro-ecology and the potential resources available, the National Breeding Committee has recommended breeding strategies for different agro-ecological regions. The description below is a very simple guide prepared on the basis of these recommendations (Table 17.1).

Plate 4 – Friesian

Black and white animal, bred in temperate regions (North America, Europe), with large body frame. Has the potential to produce 5000-10000 kg of milk per lactation. However, they are able to survive, reproduce and produce well only in cool, wet climatic conditions (hill and mid-country) and on high quality feeds. Very susceptible to tick-borne diseases (Babesiosis, Ehrlichiosis) and metabolic disorders (milk fever, ketosis). Pure Friesians are maintained in NLDB Farms at New Zealand, Ambewela and Bopathalawa.

 Plate 5 – Jersey	Light to dark brown animal with small body frame, bred in temperate regions (Europe, N. America). Very docile and easy to handle. Has the potential to produce 3000-6000 kg of milk per lactation with high fat content. Performs well under cool, wet climatic conditions and on high quality feeds. In contrast to Friesians, can adapt to moderately hot and humid conditions (such as in the coconut triangle), provided they are fed well. Pure Jerseys are maintained in NLDB Farm at Dayagama.
 Plate 6 - AFS (Australian Friesian Sahiwal)	A synthetic breed, developed in Australia by cross-breeding Friesian and Sahiwal. Stocky animal, wide variety of skin colour (black, brown with or without white patches). Has the potential for moderately high milk production (4000-5000 kg of milk per lactation) with high butter fat content and ability to perform under a wide variety of climatic conditions (wet as well as intermediate zones), provided they are fed well. AFS semen is produced at the Central Semen Processing Centre at Kundasale and is available at VS offices. This breed is recommended for cross-breeding of extensively managed stock.
 Plate 7 – Sahiwal	Brown to reddish in colour, bred in the Indo-Pakistan sub-continent. Medium sized body frame with well developed dewlap. Has the potential for moderately high milk production (2000-4000 kg milk per lactation) with a high butter fat content. Able to survive, reproduce and perform under a wide variety of climatic conditions (wet, intermediate and dry zones). Resistant to tick borne diseases. Pure Sahiwals are maintained on NLDB farms at Nikaweratiya, Ududaddawa, Polonnaruwa and Melsiripura.

17.6 Role of veterinarian, AI technician and the farmer in genetic upgrading programmes

1. The veterinarian, AI technician and farmer must always adhere to the breeding recommendations applicable to the particular area. The veterinarian and AI technician must discuss with the farmer, the choice of semen that should be used on his animals. The farmer must be made to understand that if a wrong type of semen is used on his animals, the progeny resulting from insemination may not be able to adapt to the environment of the area, resulting in stunted and unproductive animals.

2. The veterinarian and the AI technician must encourage farmers to maintain records of events in their farms such as calving dates, service dates, type of semen used, milk production, etc. This will enable the farmer, the veterinarian and the AI technician, to decide on the right type of semen to be used on the farmers' cows. Besides this will be useful in deciding on the type of veterinary interventions that may be necessary to rectify the breeding problems.

3. The veterinarian and AI technician must take a leading role in rigidly implementing the breeding programme. They must be involved in the selection of both males (or semen) and females for desirable productive traits. They must decide on the breeding value of semen to be used in the particular area and ensure that the cows and heifers are served with bulls or semen of higher breeding value (see Box 17.3 for explanatory notes on breeding value). Encourage the farmers to monitor the growth rates of the progeny and to keep only the progeny of high producing dams, showing higher growth rates and other desirable traits (e.g. body conformation and temperament) as replacement heifers in the smallholder herds.

Table 17.1 Breeding recommendations for cattle

Agro-climatic Zone	Management System	Breeding Recommendations
Wet zone hill country	Intensive system	Continuous grading up to temperate breeds (e.g. Friesian or Jersey) to raise temperate blood level to near 100%.
	Extensive system	Crossing with temperate breeds (Friesian or Jersey) to produce first generation (F_1) with 50% temperate blood. F_1 is then mated with another F_1 (of the same breed or different breed) to maintain temperate blood level at 50%.
Wet zone mid country	Intensive system	Continuous grading up to temperate breeds, to raise temperate blood to near 100%.
	Extensive system	Crossing with temperate breeds to produce F_1 with 50% temperate blood level. Thereafter cross to F_1 cross-bred bulls or bulls of AFS or AMZ.
Wet zone low country	Intensive system	Crossing with temperate breeds to produce F_1 with 50% temperate blood level. Thereafter cross to F_1 cross-bred bulls or bulls of AFS or AMZ.

	Extensive system	Continuous grading up or crossing to Indian Zebu breeds (e.g. Sahiwal) to raise exotic zebu blood levels to near 100%.
Dry zone	Intensive system	Crossing with temperate breeds (e.g. Friesian or Jersey) to produce F_1 with 50% temperate blood level. Stabilize temperate blood level at 50% by mating to F_1 bulls or bulls of AFS or AMZ.
	Extensive system	Continuous grading up with Indian zebu bulls to raise exotic zebu blood level to near 100%.
Coconut triangle	Intensive system	Crossing with temperate breeds (e.g. Jersey or Friesian) to produce F_1 with 50% temperate blood level. Stabilize temperate blood level at 50% by mating to F_1 bulls or bulls of AFS or AMZ.
	Extensive system	Continuous grading up with Indian zebu (e.g. Sahiwal) to raise exotic zebu blood level to near 100%.
Jaffna peninsula	Intensive system	Continuous grading up to temperate breeds (e.g. Friesian or Jersey) to raise temperate blood level up to 75% or more.
	Extensive system	(a) Continuous upgrading with Indian zebu breeds to raise exotic zebu blood level to near 100%. (b) Stabilize blood levels of existing temperate cross-bred animals (i.e. at 50% or above) by mating to good bulls of same population or by mating to bulls of AFS or AMZ.

Box 17.3 Explanatory notes on the use of breeding value of bull/semen and cows/heifers in the selection of bull/semen in upgrading programmes

The actual genetic potential of a cow or a bull for milk production or fat production is called *the breeding value*. This cannot be measured directly. But it can be estimated based on the performance of the animal and its relatives or offspring. The *estimated breeding value* (EBV) of an animal is the difference between the average milk production of the animal and the average milk production of animals of the same breed managed under similar conditions. Example on how to calculate the EBV for a cow and the expected performance of its calf is given below.

1. Calculation of EBV of a buffalo cow

If the average milk production of a Murrah cow in the Coconut Triangle is 2200 litres in a 305 day lactation period, and the average milk production of all Murrah cows in the Coconut Triangle is 1900 litres / 305 lactation period, then the EBV for milk production of the cow is + 300 litres (i.e. 2200-1900 litres). The estimated breeding values of the dam and the bull could be used to predict the milk production potential of the offspring. The predicted value of the calf is called the *Expected Progeny Difference* (EPD). An example of the procedure to calculate the EPD of a calf from the known EBVs for the dam and the sire is given below.

2. Calculation of EPD of a calf

Assume that the average milk production of the cows in the area	=	1900 lts (a)
and the EBV (for milk) of the dam	=	+ 300 lts
Assume that the EBV (for milk) of the sire	=	+ 800 lts (given with semen)
Then (i) the contribution of the dam to the expected progeny difference of the calf	=	½ x 300 = 150 lts (b)
(ii) the contribution of the sire to the expected progeny difference of the calf	=	½ x 800 = 400 lts (c)
Then the EPD of the calf is b+c	=	150 + 400 lts
	=	550 litres (d)

3. Expected performance of the calf

The expected performance of the calf will be the sum of the average milk production of Murrah cows in the Coconut Triangle and the EBV of the calf (a plus d) which is 1900 + 550 = 2450 for a 305 day lactation period.

Therefore in making the decision on the choice of the bull to mate this cow, the farmer should select a bull which has a higher EBV than the cow. In doing so, he should bear in mind that he should not select a sire with a very high EBV. The reason for this is that the low producing cow will not be able to produce sufficient milk to satisfy the higher milk requirement of the calf, necessary to support the higher growth rate of the superior calf

Selected references

Buvanendran, V.and Mahadevan, P. (1977) *Cross-breeding for milk production in Sri Lanka.* FAO Animal Production and Health Paper 1, Food and Agricultural Organisation, Romer, Italy. Pp. 48-54.

Jalatge, E.F.A. (1986) National breeding policy for cattle in Sri Lanka. In: *Dairy Development in Sri Lanka. (Eds. A.J.Nell and J.A. de S. Siriwardene)* pp. 40-48.

Mahadevan, P. (1951) Genetic improvement in milk yield due to selection in a herd of Sinhalese cattle. *Tropical Agriculturist. Tropical Agriculturist.* **107**, 161-167.

Mahadevan, P. (1952) The performance of the Sinhalese cattle. *Tropical Agriculturist.* CVII, (1-4) 233-238.

Mahadevan, P. (1953) Cross-breeding experiences with Sinhala cattle at Karagoda-Uyangoda. *Tropical Agriculturist* **109**, 123-127.

M/AL&F (1994) *The national breeding policy guidelines for livestock in Sri Lanka.* Ministry of Agriculture, Lands and Forestry, Colombo, Sri Lanka.

Wijeratne, W.V.S. (1970) Cross-breeding Sinhalese catle with Jersey and Friesian in Ceylon, *Animal Production.* **12**, 478-488.

CHAPTER 18

Diseases Caused by Bacteria and Viruses

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Introduction

Although the list of diseases caused by bacteria and viruses is very long, only the most prevalent diseases in Sri Lanka will be covered in this chapter. The material is arranged in two parts; Part I covers the diseases caused by viruses and Part 2 covers the diseases caused by bacteria.

Part I. Diseases caused by viruses

(a) Foot and mouth disease
(b) Bovine ephemeral fever
(c) Rinderpest
(d) Rotavirus infection

18.1 Foot and Mouth disease

Foot and mouth disease (*syn.* aftosa, aphthous fever) is a highly infectious, acute viral disease of all domesticated and wild cloven-hoofed animals. Equines are not susceptible to the natural infection. The disease is characterised by fever and the appearance of vesicles in the mouth and muzzle, feet, on the udder and teats. Death is rare except in very young animals. Foot-and-mouth disease (FMD) is of major economic importance as it spreads rapidly through susceptible species causing losses in production, particularly milk.

18.1.1 Aetiology

There are seven immunologically designated serotypes of the virus *viz.* O, A, C, SAT 1, SAT 2, SAT 3 and Asia 1. Several strains within each serotype are known to exist. The types reported in Sri Lanka include types O and C with the former being more common.

18.1.2 Occurrence

FMD is endemic in most Asian and South American countries as well as in parts of Africa. The disease has never been reported in a few countries such as New Zealand, and Japan, while it has been eradicated from many countries of Western Europe, the USA, Canada and Australia.

18.1.3 Transmission

In the tropics, FMD mainly spreads by direct contact between animals. Other modes of spread include the movement of contaminated animal products e.g. meat, milk, semen, skins and the spread on formites, vehicles and by people e.g. contaminated

fingernails of animal handlers who nose-restrain animals. Veterinarians handling infected animals carry the risk of spreading the infection to other animals if the latter are handled within 24 hours. The disease can also be spread by wind, and if conditions are favourable (high humidity and low temperature) the spread can occur over a considerable distance. The excretion of the virus from infected animals up to 4 days before vesicles appear is an important consideration in the epidemiology of the disease.

18.1.4 Routes of infection

Ruminants most often acquire the infection through the respiratory tract as it is the most sensitive route of infection. Experimental transmissions have demonstrated that the dose of virus required to infect *via* the respiratory tract is a fraction of that required *via* the oral route. The incubation period is generally 2-10 days but this could be shorter during the spread of the infection within a farm where animals could be infected by different routes.

18.1.5 Clinical features

The first signs of the infection after the incubation period include dullness, depressed appetite, fever and a marked drop in milk production. There is also the presence of blanched roughly circular areas on the tongue or dental pad. This is followed by profuse salivation with characteristic drooling of saliva and the presence of vesicles (1-7 cm) in the nostrils, muzzle, tongue, cheeks and lips. Movement of the lips gives a characteristic smacking sound. On examination of the mouth, most often the vesicles are broken and the raw underlying tissue surrounded by shreds of epithelium becomes evident. Vesicles develop on the coronary bands of the foot, the bulbs of the heels and the interdigital clefts. These lesions cause swelling and pain leading to lameness. The oral lesions heal rapidly but those in the foot may be delayed if secondary bacterial infection occurs. Lactating cows often develop vesicles on the teats and udder and therefore are predisposed to mastitis. There is abortion in pregnant animals. Mortality is rare in adult animals but deaths could occur in young animals due to acute necrotising myocarditis. Cattle that recover from FMD are immune for 2-5 years to the homologous strain.

18.1.6 Diagnosis

Salivation and lameness occurring at the same time with the appearance of vesicles should be regarded as FMD until proven otherwise. It is essential that samples are collected to confirm and identify the serotype. Vesicular fluid and epithelial fragments from ruptured vesicles should be forwarded to the laboratory in glycerol-saline in a thermos flask packed with ice in order to maintain a temperature of 4°C or less. Laboratory tests are primarily based on an ELISA where the suspected material (antigen) is tested against antibodies to the 7 serotypes of FMD.

18.1.7 Control

In most developing countries, prevention of FMD is brought about through

vaccination; the slaughter policy adopted in developed countries is not practised for economic reasons. Many countries prepare the vaccine to the strains present within the country and depending on the number of serotypes incorporated, the vaccine could be mono-, bi-, or trivalent. Some countries import the vaccines from manufacturers to cover the strains prevalent. Currently, Sri Lanka imports vaccine and the schedule prescribed includes a primary vaccination at 4 months of age followed by second and third vaccinations at 5 and 7 months, respectively. A booster is given at 12 months and thereafter is followed by annual boosters.

In animals affected by FMD, attempts must be made to minimise secondary bacterial infection, which often delays healing of the lesions. In this regard, the application of copper sulphate or alum solution on oral lesions and Stockholm Tar on foot lesions has been found to be helpful. It is essential that the vaccination programmes are coupled with measures such as the restriction of the movement of people, animals and animal products from infected areas.

18.2 Rinderpest

Rinderpest (*syn.* cattle plague) is a contagious, acute and sometimes, subacute disease primarily affecting cattle, buffaloes and pigs although other cloven-footed animals are susceptible to the disease in varying degrees. The disease is characterised by fever, necrotic stomatitis and gastroenteritis.

18.2.1 Aetiology

The disease is caused a virus of the Morbilli virus subgroup of the Paramyxoviruses and shares antigens with other members of the group *viz.* canine distemper, human measles and peste des ruminants viruses.

18.2.2 Occurrence

The disease exists in many parts of Africa, particularly, countries of North Africa but in Asia, it is confined to a few countries. The disease was eradicated from Sri Lanka in the nineteen forties but it was reintroduced in 1987 by unauthorised importation of goats from India. The mortality rates in rinderpest is quite variable ranging from 20 to 90 per cent and is thought to be due to strains of varying virulence. The wide variation in innate resistance has also been recognised as a reason for the variation in the mortality pattern.

18.2.3 Transmission

The primary mode of transmission is through direct contact between sick and healthy animals and the main site of virus entry into an animal is through the naso-pharyngeal mucosa. Infected animals excrete the virus in the expired air, nasal and oral secretions and in faeces.

18.2.4 Clinical features

The incubation period may vary from 3 to 15 days, being longest in animals with innate immunity. The clinical signs of rinderpest in cattle or buffaloes are essentially the same with the clinical reaction varying from that of a peracute disease to one that is inapparent depending on the virus strain and resistance of the animal. In the peracute form of the disease, clinical signs are limited to sudden fever, collapse and death within a few days.

The acute clinical reaction is more common when epidemics occur among animals with low innate immunity. Here too, the first clinical change is fever that persists for a few days but the animal may look remarkably normal. This is followed by congested mucus membranes, depression and a loss of appetite. Later, there is ocular nasal discharge that is watery at first, then progresses to become mucopurulent and profuse. A few days after onset of the disease, necrotic debris sloughs leaving raw sharply demarcated shallow erosions in the mouth and the adjacent structures such as the muzzle, margins of the lips and ventral tip of the tongue. The breath becomes foetid and animals are dyspaenic.

Diarrhoea follows a few days after the appearance of the mucosal lesions. At first, the faeces are watery, later there is intense straining and spasms that expose the congested rectal mucosa. The faeces then darkens, contains blood, mucus and bits of necrotic epithelium. The smell is foetid. There is severe dehydration followed by death.

Most animals with frank clinical signs die within 6 to 12 days after the onset of the disease. Some linger on for a few more weeks, while a few survive. Pregnant animals abort. The subacute form of the disease is more common in areas where the disease is endemic and where animals posses a high degree of innate immunity. The clinical signs are similar to the other forms but are transient. Skin lesions may occur as macules (circumscribed areas of discolouration). Most animals going through the subacute form recover and convalescence is short.

18.2.5 Necropsy findings

The carcass is dehydrated, emaciated and foetid. The gross lesions are largely confined to the alimentary tract. Mucosal erosions involve the pharynx and the anterior oesophagus. There are mucosal erosions of the abomasum, while the payers patches of the intestines are haemorrhagic, swollen and necrotic. The caecum, caecal tonsil, colon, and rectum are congested and often ulcerated. The congested capillaries stripe the longitudinal folds of the mucus membrane of the colon and are referred to as "zebra makings" or "tiger stripes". The heart may have subendocardial haemorrhages of the left ventricle. Subepicardial haemorrhages may be present at the base of the heart and along the coronary grooves. The most remarkable microscopic change is the destruction of the lymphocytes in the germinal centres lymphoid tissue.

18.2.6 Diagnosis

A provisional diagnosis is based on the history, particularly the introduction of

suspicious animals, the clinical signs and characteristic necropsy findings. Confirmation requires the isolation and identification of the virus, detection of specific antigens or the demonstration of a rise in antibodies in paired sera.

The specimens required for laboratory examination includes blood in EDTA, lymph node and spleen taken from a febrile animal in the mucosal phase of the disease. A few animals should be bled during the acute phase of the infection and the survivors should be bled again for convalescent serum. Histopathology with syncytia formation, the presence of intra-nuclear inclusion bodies in the cortex and necrosis of lymphocytes within the germinal centres of the lymphoid tissue provide good supportive evidence of rinderpest.

18.2.7 Immunology

Overtly clinical rinderpest confers life-long immunity in recovered animals. Calves born to immune animals are protected by the colostral antibodies. The duration of the passive immunity varies from 4 to 12 months, being longest in calves born to dams that have recently vaccinated or calved.

18.2.8 Control

The rinderpest virus is very fragile and is easily inactivated by heat and common disinfectants. Live vaccines prepared from the culture attenuated Kabette 'O' strain of rinderpest confers life-long immunity and is widely used in control programmes. The only drawback of this vaccine is the necessity to maintain an expensive cold chain.

18.3 Rotavirus infection

Rotaviruses are widely known as a major cause of diarrhoea in young and new born animals.

18.3.1 Aetiology

The causal virus belongs to the *Rotavirus* genus of the Reoviridae family of double stranded RNA viruses. There are many serotypes, most of which are host-specific. Rotaviruses are particularly prevalent on premises where animals are reared intensively.

18.3.2 Species affected

Rotaviruses have been detected in diarrhoeic calves up to 2 months of age but older calves may also be affected and excrete the virus. Rotaviruses are important pathogens causing diarrhoea in new born and young animals. Experiments in cattle calves have shown that the virus infects mature enterocytes, resulting in structural changes of the intestinal mucosae with consequent functional alterations such as maldigestion and malabsorption. Clinical disease is age-related often occurring in young animals between 1 week and 8 weeks of age. Infections in adult animals are

clinically inapparent.

18.3.3 Transmission

The virus is excreted in the faeces of infected animals and may survive in faeces for many months. Faecal contamination of water is an important mode of spread.

18.3.4 Clinical features

The incubation period is less than 24 hours. The virus destroys the enterocytes of the duodenum and ileum which are then replaced by cuboidal cells without a brush border resulting in malabsorption, maldigestion and eventual diarrhoea which causes severe dehydration and death. Most animals recover within 3 to 4 days but the absorptive capacity remains impaired for several weeks.

18.3.5 Diagnosis

Clinical signs and necropsy lesions together with age-related incidence are not specific. Diagnosis requires the detection of the virus in the faeces by polyacrlyamide gel electrophoresis. ELISA kits commercially available use the antigen-capture technique. Serological testing for antibodies will help to identify infected herds in endemic areas.

18.3.6 Differential diagnosis

In addition to dietary-induced gastroenteritis, several pathogens are responsible for diarrhoea in new-born animals. These include salmonella, enteropathogenic strains of *E. coli* and chlamydia.

18.3.7 Control

The containment of an outbreak of rotavirus infection should take into account management practices to minimise exposure of susceptible animals to the infection. This includes cleaning and disinfecting calf holding areas or calf pens with preparations such as 0.25% formaldehyde or 1% sodium hypochlorite, as well as isolating sick calves. Mixing of calves of different age groups should be avoided. Early colostrum intake is vital in increasing the resistance to the infection.

18.4 Bovine Ephemeral Fever

Bovine ephemeral fever (*syn.* three-day sickness, bovine epizootic fever) is an arthropod-borne disease in cattle and buffaloes that is characterised by transient fever, muscular pain, lameness and rapid recovery.

18.4.1 Aetiology

BEF virus is an RNA virus within the Rhabdoviridae family. The virus is pH-labile and is activated with pH changes in muscles following death.

18.4.2 Occurrence

BEF virus infection is widely reported among cattle and domesticated buffaloes in the tropical countries of Asia and Africa. In susceptible populations, the disease can cause an infection of epizootic proportions where the morbidity could be around 100 per cent but the mortality is usually less than 1 per cent. In an enzootic area, the condition is sporadic with morbidity between 5 and 10 per cent.

18.4.3 Transmission

The BEF virus is transmitted by several species of *Cullicoides* mosquitoes.

18.4.4 Clinical features

Affected animals may show rigor, watery eyes, swelling of eye lids, lameness, difficulties in swallowing, febrile reaction, inappetance and stiffness of muscles for a short period followed by spontaneous recovery within a few days. The adoption of peculiar postures including that observed in parturient paresis has been recorded in ephemeral fever.

18.4.5 Treatment

Treatment is symptomatic but can include antibiotics for secondary complications; salicylates will help to relieve muscle stiffness. Drenching should be avoided because of the high risk of aspiration pneumonia.

18.4.6 Control

Insect control is the best strategy to contain the disease but success is questionable.

Part II. Diseases caused by Bacteria

- Anthrax
- Actinobacillosis
- Actinomycosis
- Brucellosis
- Clostridial infections
  - Black quarter
  - Malignant oedema
- Haemorrhagic septicaemia
- Leptospirosis
- Johne's Disease
- Tuberculosis
- Infectious bovine Keratoconjuctivitis

18.5 Anthrax

Anthrax is a peracute or acute soil-borne infection of domestic and wild animals characterised by sudden death due to bacteraemia and toxaemia. Cattle, sheep and goats are most susceptible, followed by water buffalo, camels and equines. Pigs tend to be more resistant and carnivores even more so. Anthrax is a zoonosis. Woolsorters' disease or inhalation anthrax refers to the human disease, which was indeed a serious industrial hazard in Europe in the latter part of the 19th century.

18.5.1 Aetiology

Anthrax is caused by *Bacillus anthracis*, a Gram-positive, straight, square-ended rod. It is normally encapsulated. Under suitable conditions (presence of oxygen, temperature 20-40 °C, humidity above 60%) spore formation starts within a few hours of excretion from the animal body. The spores are very resistant and will remain viable for years.

18.5.2 Occurrence

Anthrax has a world-wide distribution with the major naturally occurring areas being in the tropic and subtropics of Africa, South America and Asia. The rapid production of resistant spores in warm climates leads to contamination of soil and water. Outbreaks of the disease occur when animals return to contaminated pastures or watering points, often coinciding with the local rainy season. Epidemics occur when an anthrax carcass is opened up by predators such as carnivores, and widespread contamination of soil in the surrounding area takes place. Sporadic cases occur when spores are dispersed by the wind and predators, *via* contaminated feed, fertilisers of animal origin and tannery effluent. In Sri Lanka, the last case of anthrax was reported in sheep in 1969.

18.5.3 Pathogenesis

The route of infection is usually by ingestion of spores of *B. anthracis*. These appear to enter the gut and germinate in the tissues to produce vegetative bacteria. The organism multiplies in the blood stream. They resist phagacytosis because of the presence of the capsule. When a sufficiently high concentration of the organisms are reached in the blood, a protein produced affects the respiratory enzymes within cells

which causes death of the animal. Spore formation does not occur until the vegetative bacteria is exposed to air.

Anthrax is an occupational hazard among stock owners and people working with wool, hides and skins, infection occurring through skin abrasions or aerosols.

18.5.4 Clinical features

In cattle the disease is peracute or acute and usually occurs in adult animals. Anthrax essentially produces a highly lethal bacteraemia where death follows within a few minutes to several hours in apparently healthy animals. In the peracute disease which lasts only a few hours, there is pyrexia (108°F), muscle tremor, tachypnoea and occasional convulsions. In the acute syndrome, the clinical signs are similar to that in the peracute form, together with the passage of fresh blood in faeces.

In anthrax, blood fails to clot properly and after death dark tarry blood often exudes from the natural orifices and through the skin. Bloat and decomposition occur rapidly. A carcass suspected of anthrax should not be opened but if accidentally opened, indicates septicaemia. The spleen is often greatly enlarged.

18.5.5 Diagnosis

In tropical countries, the possibility of anthrax should be suspected in all cases of sudden death or where subcutaneous oedema and throat swellings are seen. Differential diagnosis should include blackleg, enterotoxaemia, pasteurellosis, lightning strike, snake bite and plant poisoning.

A blood smear should be made from a dependant part e.g. the lower part of the ear. The wound should be plugged with disinfectant soaked cotton wool. The smear should be heat-fixed immediately and stained with Giemsa. The polychrome methylene blue method is quicker but less efficient with putrefying cadavers. Large-square-ended blue bacilli arranged in short chains and surrounded by a purple staining capsule are seen in positive cases.

Tissues or fluids should be submitted for culture or animal inoculation, if smears are inconclusive. A piece of cartilaginous rib or skin may be useful if other tissues are not available. Tissues are best preserved by refrigeration or immersion in 50% glycerol saline.

18.5.6 Treatment

Anthrax is a notifiable disease but treatment of the pyrexic animals can be carried out. *B. anthracis* is susceptible to penicillin, tetracyclines and some sulphonamides. Penicillin is probably most effective and should be given in large doses, 10,000 units/kg crystalline penicillin twice daily for at least 3 days. Drugs are only effective when treatment is started early in the course of the disease. In the face of an outbreak the body temperatures of valuable animals should be monitored for at least two

weeks, and antibiotics given to those with fever. Animals must be revaccinated after the effects of the antibiotics have worn off.

18.5.7 Control

In endemic areas and in places known to be infected, bovines should be protected by annual vaccination. The most widely used vaccine is the Sterne's spore vaccine which is prepared from a live attenuated strain of the organism. Immunity wanes within 9-12 months and vaccination is therefore carried out a month before the peak risk period starts. Continued vaccination over a 4 to 5 year period eliminates some foci of infection but in other places infection persists for much longer. In some areas, one species of animal tends to be affected and not others; selective vaccination may be appropriate.

When anthrax is diagnosed, it is imperative that access to the carcass is prevented. Pending disposal, guards, thorn bushes should be used to prevent predators from tearing open the carcass. In the tropics, anthrax organisms die within a few days in an unopened carcass. Burning of the carcass should only be carried out if it can be done effectively. If not, the carcass should be buried 1-2 m deep and covered with lime. The contaminated area around the carcass should be burnt. Surfaces of contaminated objects should be treated with 10% caustic soda, 10% formalin solution or other approved disinfectant.

18.6 Actinobacillosis

Actinobacillosis is a chronic disease characterized by the presence of granulomatous lesions in the tongue, pharynx and associated lymph nodes. Lesions in the tongue give rise to the syndrome known as "wooden tongue", which causes anorexia and interference with swallowing occurs.

18.6.1 Aetiology

The diseases is caused by the Gram-negative aerobic bacterium, *Actinobaccillus lignieresi.* Other organisms such as *A. pyogenes* may be present in the lesions.

18.6.2 Occurrence

The disease occurs sporadically and is most common in animals between 18-36 months of age.

18.6.3 Pathogenesis

Actinobacillus lignieresi is a normal inhabitant of the oral cavity. The infection develops following the entry of the organism into the tissues, usually following trauma or abrasions from rough, course herbage (twigs, rough grass, stalks). The organism enters the tissues at the site of injury forming pus and eventually a granuloma. Healing of the lesion is by fibrosis and is often very slow.

18.6.4 Clinical findings

The clinical signs set in quickly resulting in the affected animal being unable to swallow and may be anorexic. Cudding may be present with excessive salivation. Examination of the tongue is resented but if examined the base of the tongue is swollen with discharging pus. The pus may contain "sulphur granules", which appears as gritty whitish-yellow granules when washed in saline. In chronic cases the tongue is immobile and there a is marked loss of condition.

18.6.5 Diagnosis

Clinical signs of actinobacillosis where the tongue is involved is fairly distinctive. However, care should be taken to look for the presence of foreign bodies which may also present similar clinical signs, especially excessive salivation.

18.6.6 Treatment

Sodium iodide (1g/12 kg body weight) given intravenously as a 10 per cent solution is recommended. Penicillin, streptomycin and tetracyclines may also be used, and may be more convenient, particularly in non-lactating animals. Five intramuscular doses of streptomycin is widely used and has been found to be very effective.

18.6.7 Control

Isolate animals with discharging lesions to prevent contamination of feed with the discharges.

18.7 Actinomycosis

The lesions of actinomycosis occur in the bones of the jaw to produce the condition known as "lumpy jaw". The resulting lesion interferes with eating and eventually causes death by starvation.

18.7.1 Aetiology

Actinomycocis is caused by *Actinomyces bovis*, a gram-positive filamentous anaerobic organism which is a normal inhabitant of the bovine mouth. Lesions may often be infected by a wide variety of other organisms.

18.7.2 Occurrence

The disease is most often seen in mature animals.

18.7.3 Pathogenesis

The organism gains access to the gum and the underlying tissues through abrasion of the mucosa or eruption of a tooth. A granulomatous lesion develops causing rarefaction and osteomyletis. The lesions are often heavily encapsulated in fibrous

tissue in which pockets or larger areas of pus may be found. The pus contains yellowish sulphur granules.

18.7.4 Clinical findings

Most lesions are noticed as hard, painless swellings on the jaw bone at the level of the first molar or premolar teeth. They may enlarge rapidly causing severe pain on palpation or grow very slowly. Sinuses may erupt through the skin and discharge yellowish pus with hard "sulphur granules". Enlargement of the jaw bone, tooth loss or loosening may cause dysphagia and progressive emaciation occurs over a period of time.

18.7.5 Diagnosis

The bony lesions are characteristic and diagnosis of lesions of the rami of the mandible is easy. When discharging sinuses are present, smears and culture of the organism will help the confirmation of clinical diagnosis. Smears made from the pus or crushed sulphur granules contain masses of Gram-positive branching filaments and cocci. Cuture of the lesions often yields a mixed growth of *A. bovis* and organisms such as *A. pyogenes*.

18.7.6 Treatment

Penicillin therapy repeated daily for 3-5 days may be effective as with other antibiotics and iodine treatment mentioned under actinobacillosis. Large lesions may require prolonged treatment and bony changes may never resolve. Many cases, especially those with secondary dental problems (loss, loosening etc.) frequently lose weight rapidly and never respond to treatment.

18.8 Brucellosis

Brucellosis is the disease produced by infection with organisms of the genus *Brucella* that affects the reproductive organs of animals. The disease also affects man.

18.8.1 Aetiology

Bovine brucellosis is most frequently caused by *Brucella abortus* of which nine biotypes are recognized. *Br. melitensis* infection may occur if cattle or buffaloes are grazed with infected goats or sheep.

18.8.2 Occurrence

Brucellosis is recognized in many parts of the world where cattle are kept, particularly in areas of intensive cattle raising. Brucellosis of bovines is primarily a disease of sexually mature females, with bulls and immature animals often showing little or no clinical disease.

18.8.3 Pathogenesis

Animals usually acquire the infection through ingestion of contaminated pasture and water or by licking the discharges, placenta or a new born calf. Transmission can occur through the conjunctiva or through inhalation. The infected pregnant cow or heifer is the most important source of *Br. abortus*. The foetal membranes and foetal fluids contain volumes of bacteria varying from 10^{12} to 10^{14} organisms that contaminate the vulva, perineum, legs and tail, as well as the surrounding environment. The excretion of the organisms may occur a few days before the infection and indeed for a varying period of time after the abortion. Brucella organisms can survive in contaminated material for days if not weeks in the shade and when protected by the foetus. Predators can move a foetus over long distances, thus help in disseminating the infection. Over 90% of cows and heifers excrete the organism in the colostrum and milk during the first month of lactation. Thereafter the excretion is intermittent. Calves may get infected *in utero* or by suckling infected dams. Venereal transmission is rare in bovine brucellosis but AI with infected semen often results in transmission.

18.8.4 Clinical findings

The clinical course in brucellosis is largely dependent on the age at which the animal is infected rather than on the dose or the virulence of the organism.

(a) Calves that are infected prior to puberty lose the infection once removed from the source of contamination. However, infected heifer calves may retain the infection until maturity.

(b) Infection acquired after puberty is likely to be retained almost permanently. Following entry through the mucous membrane, the organism multiplies in the local lymph nodes. A subsequent bacteraemia ensures the colonization of the organism in predilection sites such as the mammary gland, testes, seminal vesicles and associated lymph nodes. Other sites include the joints, bursae, liver and spleen. It is only during the latter part of pregnancy when the placenta develops that the organism invades the uterus producing the classical signs of brucellosis. The incubation period is around 6 weeks and abortion follows from the 7^{th} month of pregnancy onwards. In less severe infections, cattle may give birth for a full-term weak calf or merely show retained placenta. In these cases endometritis is likely to follow as a sequel to chronic metritis.

(c) Mature non-pregnant animals or animals in early pregnancy when exposed to the infection are more likely to develop immunity than show signs of the disease.

(d) Infected males show no clinical signs apart from testicular enlargement. The semen may contain pus. The infection may or may not affect fertility. Infected males and females may show evidence of hygroma which in certain countries is highly indicative of the infection.

18.8.5 Necropsy findings

Lesions are not specific to brucellosis. Necrotic placentitis is a frequently observed lesion. In bulls, seminal vesiculitis, epididymitis and orchitis may lead to abscess formation. In the udder, the lesions seldom progress to cause clinical mastitis but cell counts are likely to increase.

18.8.6 Diagnosis

Brucellosis as a cause of abortion can be confirmed by demonstrating the organism in culture or on smears. The modified Ziehl-Neelsen or Koster methods are useful in demonstrating the organism on smears prepared from the placenta, foetal stomach contents or vaginal discharges. Tissues for culture may include foetal stomach contents or lung or placenta, vaginal discharges, milk and semen. Necropsy samples include mammary tissue and supramammary or iliac lymph nodes.

Control and eradication programmes rely on serological tests for detecting infected animals. The screening tests include the Rose Bengal and card tests carried out on drops of serum or whole blood and the milk ring test made on individual milk samples or from bulk milk tanks. ELISA is now widely used as a screening test. These tests are sensitive but lack specificity. The Complement Fixation Test (CFT) is by far the most specific and sensitive test although it is a cumbersome test which also requires considerable technical skill. The Serum Agglutination Test (SAT) is easier to carry out but is less specific and sensitive compared to the CFT and is difficult to interpret in herds where S19 vaccine has been used.

A single negative result should not be considered as evidence of the absence of infection as the sample may have been taken when the animal was incubating the disease. Therefore the negative samples should be followed by a second test which should be done 30-60 days later or in the case of animals that calve, 14 days after the calving.

18.8.7 Control

The control of brucellosis is affected through combination of good hygienic management practices and vaccination. Pregnant animals must be kept under observation and isolated if there is a possibility of abortion. Ensure that all uterine discharges and placental membranes are well disposed of. Vaccination has been found to be the most appropriate method of control, particularly in countries with a high prevalence of the brucellosis. At least three clear tests should be negative before a herd is declared disease-free.

18.8.8 Public health

Man may acquire the infection by direct contact with cows at abortion, parturition or during the post-partum period. The splashing of infected droplets into the eye is an important mode of spread to man. Infection also occurs following the consumption of

unpasturerized milk or dairy products. The clinical signs in man include recurrent fever, headache, muscle and joint pain and general weakness.

The use of disposable plastic gloves and the disinfection of the vulva, vagina and tail during obstetrical manipulations will greatly reduce the risks of infection. Pasteurization of milk and dairy products makes them safe for human consumption. Treatment with streptomycin and tetracycline affect a cure provided the treatment is started before a chronic infection is established.

18.9 Clostridial infections

18.9.1 Black quarter

Black quarter (*syn.* Black leg) is an acute fatal disease in young cattle and buffaloes characterised by the development of crepitant swelling in the muscles of one or more limbs.

18.9.1.1 *Aetiology*: The disease is caused by *Clostridium chauvoei*, an anaerobic spore forming Gram-positive bacillus.

18.9.1.2 Occurrence: The disease occurs worldwide. It most frequently affects animals aged 6-24 months. Animals affected are usually those in good condition and on a high plane of nutrition. The disease has a sporadic occurrence but seem to be localized in certain areas or particular fields. In areas where the black quarter has not been known to occur, soil disturbances such as excavations or drainage can suddenly initiate an outbreak probably by turning up or by creating conditions necessary to activate latent spores.

18.9.1.3 *Pathogenesis*: Infection occurs following ingestion of spores from contaminated pasture. These spores are passively absorbed by the gut and accumulate in tissues where they remain dormant. Germination of the spores occur when the oxygen tension of the tissue falls as a result of injection, bruising or in the case of fat animals after heavy exertion. The bacteria produce proteolytic enzymes and ferment carbohydrates to produce gas, acetic acid and buytric acid which give rise to the characteristic muscle lesions.

18.9.1.4 *Clinical signs*: Early in the outbreak, animals in good condition may be found dead or severely ill with subcutaneous swellings. Those affected may be severely depressed, pyrexic (105-107°F) and completely anorexic. There is also tachycardia (100-120/min) and an increased respiration rate as well as complete cessation of rumination. Hot, painful swellings develop over the hindquarters, shoulders and neck. On palpation, emphysema and crepitations can be heard or felt. Usually these swellings develop in the upper part of only one limb.

If untreated, the disease is of short duration (12-36 hours) and death occurs within 48 hours. Treated animals often recover but are frequently permanently lame and need to be are culled for economic reasons.

18.9.1.5 *Necropsy findings*: Death results from the affect of the bacterial toxins on the myocardium. Bloat and putrefaction rapidly occur and the carcass becomes distended by gas. The subcutaneous tissue contains gas and gelatinous exudate. In the deeper muscles the colour varies from dark red to almost black. The affected muscles when cut appear like lung tissue owing to the presence of gas. The regional lymph nodes are swollen and haemorrhagic.

18.9.1.6 *Diagnosis*: The characteristic clinical signs and lesions on necropsy are sufficient to make a tentative diagnosis, but it must be confirmed by laboratory diagnosis. This includes the preparation of a direct smear which should reveal the presence of Gram positive rods with central or subterminal spores. The specific fluorescent antibody test provides a positive diagnosis and is the method of choice. Culture may be useful but is often contaminated by other clostridial species.

18.9.1.7 *Treatment*: This is worth attempting only in the early stages of the disease when very large doses of antibiotic need to be administered. Penicillin is the drug of choice.

18.9.1.8 *Control*: Vaccines prepared from whole cultures inactivated with formalin are effective. Most vaccines provide protection within 3 weeks of the single subcutaneous injection and a second dose may be given 6 weeks later, if a heavy challenge is expected. Protection usually persists for a year.

18.9.2 Malignant oedema

Malignant oedema (*syn.* gas gangrene) is an acute inflammatory condition of the muscles associated with profound systemic toxaemia following wound infection.

18.9.2.1 *Aetiology*: The disease is principally caused by *Clostridium septicum* but other clostridial species such as *C. chauvoei*, *C. perfringens* and *C. novyi* have also been isolated from cases of malignant oedema.

18.9.2.2 *Occurrence*: The disease has a world-wide distribution affecting all age groups of animals. Malignant oedema usually occurs sporadically affecting individual animals except under special circumstances when outbreaks may occur.

18.9.2.3 *Pathogenesis*: Malignant oedema is a soil-borne infection where the organism gains access into tissues through contaminated deeply punctured wounds often accompanying trauma. The infection may also follow vaccination and venepuncture or through the umbilical cord of newborn calves. The deep sites within tissues offer a favourable environment for the organism to multiply. The potent toxin produced at the lesion causes severe muscle necrosis and results in death when absorbed into the circulation.

18.9.2.4 *Clinical signs*: Clinical signs develop within a few hours of the infection. The affected animals are severely depressed, pyrexic (41-42°C) show muscle tremor and usually are stiff or lame. There is swelling and gelatinous oedema of the affected area which pits on application of pressure. The emphysema which also develops in the

lesions has close similarities to those present in black leg.

18.9.2.5 *Necropsy findings*: The clinical course is usually short and the animal dies within 24-48 hours after the first appearance of the clinical signs. Tissue changes occur rapidly after death, particularly in warm weather. At the site of swelling, there is extensive blood tinged subcutaneous oedema including a few gas bubbles. The oedema also extends to the muscles as well as the intermuscular connective tissue. Affected muscles are dark and the spleen is usually swollen.

18.9.2.6 *Diagnosis*: The presence of wounds and the accompanying toxaemia is suggestive of malignant oedema but the clinical signs and lesions present at necropsy may greatly overlap between those of black leg and malignant oedema. Hence, a laboratory diagnosis is essential to confirm the disease. Specimens for laboratory investigations include affected muscles (3-4 blocks of tissue each of around 2 cubic cm) and oedema fluid. Care should be taken to collect the samples aseptically, packed these into a plastic bag and sent frozen or chilled with ice to a laboratory. Guinea pig inoculations cause death of the guinea pigs and produce long chains of rods evident in liver impression smears.

18.9.2.7 *Treatment*: Massive doses of penicillin and streptomycin may be effective only if administered during the early stages of the disease.

18.9.2.8 *Control*: The practice of asepsis during vaccination and parental administration of medication is an essential step in the prevention and control of malignant oedema. The disease can indeed be prevented through vaccination specific for the disease or by using a multiple vaccines covering the common clostridial infections.

18.10 Haemorrhagic septicaemia

Haemorrhagic septicaemia (HS) is an acute form of pasteurellosis of cattle and buffaloes caused by specific serotypes of *Pasteurella multocida.*

18.10.1 Aetiology

Two serotypes of *Pasteurella multocida* namely, B:2 and E:2 (Carter–Heddleston classification) are responsible for the disease. In Asian countries, the most predominant serotype in HS is B:2, while in African countries E:2 is commonly associated with the disease. A few countries such as Egypt and Sudan have recorded the isolation of both serotypes in outbreaks of HS.

18.10.2 Occurrence

The disease is endemic in most South and South East Asian countries. It has also been reported from parts of Africa. Studies in Sri Lanka have clearly indicated that the occurrence of the disease has a bearing on the type of animal, the animal management system, the location where the disease occurs and the season. Although the disease occurs in cattle and buffaloes, epidemiological studies have demonstrated that the

latter species is more susceptible. The morbidity and mortality rates are also dependent on the area where the disease occurs. For example, outbreaks of disease in non-endemic areas can cause death in animals of all ages, while disease outbreaks in the endemic areas usually results in death of animals between 6 months and 2 years of age, as a large proportion of the adult animals possess naturally acquired immunity. The incidence of the disease is also related to animal husbandry practices where the disease has a high incidence in cattle in large nomadic herds when compared to those in small intensively managed herds. Studies in Sri Lanka and elsewhere have also indicated that outbreaks are more common with the onset of the monsoon rains and this is thought to be associated with stress imposed by climatic changes and the increased work load in paddy fields which commences around this period.

18.10.3 Pathogenesis and clinical signs

The disease is acquired following inhalation or ingestion of the organism. Owing to the very short clinical course, signs are often not noticed in natural outbreaks of the disease. Sudden death of apparently normal animals is the common complaint. Experimental studies however have been able to examine the clinical signs more closely through a protracted clinical course following intranasal infection. In the experimental model, the clinical signs have been classified into 3 phases. The initial phase is where there is an increase in temperature, depression and anorexia, followed by a phase of respiratory distress associated with mucopurulent ocular-nasal secretion, leading to a terminal phase of recumbency and death. Submandibular oedema which can extend to the brisket is also an accompanying clinical sign in HS. An atypical form of HS associated with an acute fibrinous bronchopneumonia has also been reported

18.10.4 Necropsy findings

At necropsy, the lesions will differ according to the clinical course. In animals that die following a short clinical course, the most conspicuous necropsy finding is a general haemorrhagic diathesis associated with diffuse haemorrhage in tissues such as the abomasum; petechiae and echymosis of subepicardium and endocardium. There is also a marked congestion of the lungs. In animals that die following a relatively protracted clinical course, pneumonia involving the anteroventral lobes is a classical necropsy finding. Oedema of the submandibular and brisket region may also be evident in both forms of the disease.

18.10.5 Diagnosis

A tentative diagnosis may be made on the basis of the following: the area in which the disease occurs i.e. enzootic or non-enzootic, vaccination history, the short clinical course leading to sudden death, the age of animal and the necropsy findings. Laboratory diagnosis is essential to confirm HS. The samples which need to be submitted include aseptically collected heart blood from a fresh carcass or in the case of an "old" carcass, an entire long bone e.g. femur. The bone marrow is scooped out in the laboratory and used in the isolation of the organism. Blood collected from animals in the moribund stage and nasopharyngeal swabs from in-contact animals will help to isolate the organism. All samples should be sent immediately to the laboratory

packed in ice. The identity of the HS-causing pasteurellae is based on the cultural characteristics, morphological appearance, biochemical reactions and serological tests.

18.10.6 Treatment

Treatment is effective only in the early stages of the disease, however, it is rather difficult to detect animals during the early stage of HS. The only practical way to detect those in the early stage of the infection is to monitor the rectal temperature twice daily of all in-contact animals soon after the first case is detected. Antibiotic therapy should be instituted immediately to all animals that show an increased temperature. The organism is sensitive to penicillin, ampicillin, streptomycin and oxytetracycline.

18.10.7 Control

Vaccination is the accepted method of control. The oil adjuvant vaccine which is used in many countries including Sri Lanka, is administered at a time that will ensure that immunity will be at its peak at the time of monsoon rains, when outbreaks usually occur. This vaccine confers immunity up to one year. The recommended protocol is to immunise calves above 4 months of age followed by a booster after 3 months and repeat annual vaccinations. The alum precipitated vaccine is used in the face of an outbreak and has been found to induce immunity up to 4-6 months.

18.11 Leptospirosis

Leptospirosis refers to a group of diseases in man and animals associated with infection by organisms of the genus Leptospira. Although a majority of the infections are subclinical, the organisms of this species can cause clinical signs such as fever, jaundice, anaemia, nephritis and abortion. It is also a zoonosis and in man can give rise to three syndromes namely, (a) flu-like syndrome (b) meningitis and (c) icteric syndrome.

18.11.1 Aetiology

Leptospirae of the interrogans subgroup and L. biflexa serotypes andamana and patoc have all been associated with the disease in cattle. The *L. interogans* comprises 26 serogroups and over 200 serovars.

18.11.2 Occurrence

The disease has a world-wide distribution. In Sri Lanka, serovar *Weerasinghe* (30.2%) has been most common among buffaloes, while *pomona* (26.6%), *hardjo* (24%) and *pyogenes* (11.3%) have followed with lower incidences. In regions of Sri Lanka where human leptospirosis is reported, 41.1% of the buffaloes were found to be sero-positive. The presence of serum antibodies against the organism have also been reported in cattle.

18.11.3 Pathogenesis

Urine, aborted foetuses or uterine discharges of infected animals contain viable organisms that can infect susceptible animals. Infected rodent, dog (*L. canicola*) or cow (*L. hardjo*) urine could also be an important source of infection. The most common method of transmission is through splashing of freshly voided urine from other cattle. Venereal or congenital transmission occurs but is less important. The organism gains access through abrasion or directly through the mucosae of the pharynx and multiples to give rise to a septicaemia within 4-7 days. The antibodies rise and the organism get restricted to certain sites in the brain (meningitis), kidney tubules where it is shed in urine, uterus and placenta where it invades the foetal membranes and foetus to cause abortion. Invasion of the liver causes jaundice and haemolytic anaemia.

18.11.4 Clinical findings

In calves less than two months of age, meningitis is the main clinical syndrome. Affected animals are severely depressed, anorexic, pyrexic (40-5-41.5°C) and show signs such as opisthotonus, trismus, muscle tremors and paddling movements when in lateral recumbency. Some calves may be blind. In slightly older calves (around 6 months), the clinical signs are due to septicaemia with anorexia, dullness and pyrexia. Petechiation of the mucus membranes occurs and there is haemolytic anaemia with pallor and jaundice and haemoglobinuria.

Infection in adult milking cows results in a sudden drop in the milk yield with pyrexia. However affected cows continue to eat and do not show marked changes in demeanour. Very occasionally abortion will occur at this stage. With chronic infection, following localisation in the kidneys and uterus, abortion occurs about 6-12 weeks after infection. Abortion can occur at any stage of gestation from 4 to 9 months. Following abortion very high concentrations of the organism may be excreted in the urine discharges for up to 22 days. It is also likely that the foetal membranes will be retained.

18.11.5 Necropsy findings

Few lesions are seen in animals, particularly calves that succumb to acute leptospirosis. Haemorrhages and petechiae may be present, particularly on the kidney and serosal surface and there may be evidence of haemolytic anaemia or jaundice. Histologically, the most prominent lesion is the presence of centrilobular necrosis and focal or diffuse interstitial nephritis.

18.11.6 Diagnosis

Leptospirosis may be suspected on the basis of the clinical signs or necropsy findings but laboratory investigations are essential for confirmation. These include the (a) demonstration of leptospirae by dark ground examination of urine and (b) isolation of the organism from blood, milk, urine of infected cattle. Both these procedures are difficult to carry out.

Serological tests are relatively easy and are commonly performed in diagnosis. This could be achieved by the demonstration of (a) a rising antibody titre in paired serum samples taken 14 days apart, which indicates a recent leptospiral infection and (b) an individual serum sample containing an antibody titre of 1:100 or greater to a leptospiral serotype indicating a chronic or active infection. Levels of antibody of 1:10 to 1:30 are considered not indicative of an active infection. Diagnosis of leptospiral abortion is based on foetal serology, and direct fluorescent antibody test of foetal material and culture. Dam serology is of very little value.

18.11.7 Treatment

A number of antibiotics can be used to treat leptospirosis. The elimination of the organism from the kidneys can be achieved using 3-5 daily injections of streptomycin 10 mg/kg or 25 mg/kg in a single injection. Tetracyclines and ampicillin may also be satisfactory.

18.11.8 Prevention and Control

Carrier animals should be eliminated either by treatment or blood testing and removal. The reservoir of infection, such as rats should be controlled. Hygiene, the provision of clean water supplies and fencing-off areas holding stagnant surface water, may also reduce the transmission of the disease. Vaccination is practised in some countries primarily to protect humans.

18.12 Infectious Bovine Keratoconjuctivitis

Infectious Keratoconjuctivitis (*syn.* pink eye, New Forest Disease, infectious ophthalmia, blight, infectious keratitis) in cattle is a highly infectious condition which spreads rapidly through the herd affecting the eyes, particularly of young cattle.

18.12.1 Aetiology

Although many organisms have been isolated from the infected conjunctival sac, *Morexella bovis* is recognised as the primary cause of this condition in cattle.

18.12.2 Occurrence

The disease has a world-wide distribution. All age groups of animals are susceptible but the disease is mostly seen in young animals between 6 weeks and 12 months of age. *Bos indicus* breeds of cattle show a much lower incidence of pink eye compared to *Bos taurus* breeds. Ultraviolet rays from solar radiation precipitate the disease, with most cases occurring during the dry periods experiencing many hours of sunlight. In temperate countries it is common during the summer. Flies are known to spread the disease within and between herds.

18.12.3 Clinical findings

The most common clinical sign is the presence of an ocular discharge followed by

corneal opacity or both. The condition is predominantly unilateral. At first the animal blinks frequently, and usually stands looking away from the light with the head lowered and eyes almost closed. There is a copious discharge of tears, a rapidly developing inflammation of the conjunctiva and the third eye lid. The cornea becomes opaque. After some days a yellowish–white area develops and progresses into an ulcer. Blood vessels develop across the cornea from the sides of the eye. This goes on until the entire cornea is covered by the vascular tissue producing the so-called "pink eye". Discharges containing pus occur at this time. The condition continues for three to four weeks after which recovery commences and is complete in about two weeks.

18.12.4 Treatment

Ophthalmic preparations containing antibiotic and cortisone are recommended. Eye ointments containing neomycin and hydrocortisone introduced into the conjunctival sac twice daily has been found to be effective. An antibiotic sensitivity test is recommended as strain variations in antibiotic sensitivity to *Morexella bovis* have been observed.

18.13 Tuberculosis

Tuberculosis is a chronic disease manifested by the progressive development of tubercles in any of the organs of the body.

18.13.1 Aetiology

Mycobacterium bovis is the usual cause of tuberculosis in cattle and is equally pathogenic to other ruminants, pigs and man. *M. tuberculosis* is responsible for the majority of cases in man and is occasionally isolated from farm animals. Previously it was customary to refer to causative agent as the cattle strain, the avian strain and the human strain of *Mycobacterium tuberculosis,* but those pathogenic to cattle and other animals are now referred to as *Mycobacterium bovis*. *M. bovis* is a long slender, rod shaped organism which belongs to the acid-fast group of bacilli. The organism is moderately resistant to heat, desiccation and certain disinfectants such alkalis and phenols but is readily destroyed by sunlight.

18.13.2 Occurrence

Tuberculosis is distributed world-wide but the prevalence could vary according to the type of husbandry and the efficacy of the control measures. The incidence of bovine tuberculosis is low when cattle are managed under extensive management systems. Housing of animals can aid the spread of the infection between animals. Stress imposed by repeated pregnancies and lactation, weakens the defences of the host, hence tuberculosis is more commonly seen in older dairy cattle housed under poor hygienic conditions. Tuberculosis still remains a disease of great importance although some countries such as Australia have been successful in eradicating the disease to a very large extent. Tuberculosis is seldom seen in Sri Lanka.

18.13.3 Public health

Tuberculosis is a disease of great public health concern, as man is susceptible to infection with *M. bovis* and *M. tuberculosis*. Man usually gets infected with *M. bovis* by the aerosol route or by drinking tuberculous milk. Pasteurisation effectively kills mycobacteria. Meat inspection and condemnation of affected organs or parts of organs are important measures to prevent human infection.

18.13.4 Pathogenesis

The infected animal is the main source of the infection as the organism is excreted in exhaled air, sputum, faeces, milk, urine, vaginal and uterine discharges. The entry of the tubercle bacilli can occur by various routes. Cattle are much more susceptible to infection by inhalation of contaminated dust particles and droplets than by ingestion. Milk is an important source of infection for calves. The organism may remains viable for weeks or months in shaded pasture, cattle yards and stagnant water and therefore, indirect transmission is readily achieved.

18.13.5 Clinical signs

In the early stage, there are no clinical signs except for the appearance of general apathy and malaise. Cattle with the pulmonary form of the disease develop a persistent cough, anorexia and a gradual loss of body condition. In the intestinal form of the disease, there is persistent diarrhoea and a progressive loss of weight. Infection of the mammary gland results in a hard painless enlargement of the gland that may extend to the supramammary lymph nodes. The milk from an infected udder becomes watery and contains large numbers of organisms. Despite the clinical changes mentioned, the disease in most instances is detected only at slaughter or death from other causes.

18.13.6 Necropsy findings

The lesions in tuberculosis are largely confined to the lungs and the draining lymph nodes. When compared to cattle, the lesions in buffaloes are less calcified and resemble abscesses with caseous material arranged in layers. The distribution of lesions may vary from a large, solitary abscess to widespread smaller lesions, the so-called miliary tuberculosis affecting the peritoneum, pleura and other organs. In the lungs, these military abscesses may extend to cause suppurative bronchopneumonia.

Lesions present at necropsy can vary from a single small well-calcified lesion to a large abscess containing semi-liquid pus or wide spread smaller lesions, the so- called miliary tuberculosis affecting the pleura and peritoneum. Histopathogical examination of tissues reveal a variety of structures depending whether there is exudation or necrosis. A variety of cells that include plasma cells, macrophages, epithelioid cells and giant cells could also be seen.

18.13.7 Diagnosis

In live animals, tuberculosis is diagnosed by demonstrating hypersensitivity to tuberculo-protein in the tuberculin test. Tuberculin is a purified protein derivative (PPD) produced by growing mycobacteria in a protein-free medium and precipitating a cell-free filtrate. The test is usually carried out by intradermal inoculation of tuberculin on one occasion, i.e. the single intradermal test. The site of inoculation of the skin is on the side of the back or the caudal fold. The reaction is read after 72 hours and the presence of any oedema is regarded positive. The oedema following the tuberculin test is more pronounced and extensive in the buffalo compared to cattle. It has also been noted that the skin reaction in the buffaloe persists longer than in cattle and may last up to 10 days. A small circumscribed swelling of 2-3 mm without oedema is considered negative. False positive reactions occur relatively frequently and may be caused following infection with non-tuberculous mycobacteria. Animals that have been infected may appear negative in the tuberculin test. Conversely, animals in advanced stages of tuberculosis may become anergic and also pass the test. Such cases are usually emaciated and should be detected by clinical examination.

At post-mortem, the diagnosis is confirmed by the demonstration of the organism. Suspected material should be submitted for culture or animal inoculation. Mycobacteria grows very slowly, hence the results will not be available for 4-8 weeks. Ziehl-Neelsen staining may reveal acid-fast organisms but is not a sensitive technique. Tuberculosis should be differentiated from norcardiosis, actinomycosis and parasitic granulomata which present similar lesions.

18.13.8 Treatment

Treatment is of little practical value. Attempts to treat livestock have been made on a small scale in the field but the cost of such treatment has been found to be high and not all animal treated were cured and reinfection was common. Owing to these disadvantages and the risk to human health, treatment is not recommended.

18.13.9 Control

Effective eradication can be achieved by slaughtering all tuberculin positive animals, if the economics of the livestock industry permits. Such a policy however is beyond most Asian countries. The policy may however be modified to slaughter of all clinical cases, testing of the rest of the herd and dividing the herd into reactor and non-reactor groups. The reactors could be tested repeatedly every six months and the number of animals in the reactor herd could be reduced by rearing the off-springs in clean premises. All attempts should be made to prevent spread of the infection by isolating infected animals and avoiding the introduction of infected animals to clean herds.

18.14 Paratuberculosis

Paratuberculosis (Johne's disease) is an infectious disease of ruminants characterised by a long incubation period and clinically by diarrhoea and weight loss.

18.14.1 Aetiology

Paratuberculosis is caused by *M. paratuberculosis* (*M. johnei*), a small acid fast bacillus which is relatively slow to grow in the laboratory. The organism is relatively resistant to light and disinfectants and may survive for many months in faeces, water and soil.

18.14.2 Occurrence

Johne's disease is widespread in many countries. The disease occurs sporadically and the mortality rate is usually less than 1% in infected herds, but ill health and reduced productivity, especially milk, due to the disease causes heavy economic losses.

18.14.3 Epidemiology

Young animals are very susceptible and usually become infected at an early stage but develop the clinical disease only as adults. Stress, parturition and nutritional deficiencies influence the precipitation of the disease.

18.14.4 Pathogenesis

Under natural conditions infection is by the oral route, following ingestion of the organism in water or feed contaminated by faeces of infected animals. Some clinically infected animals excrete *M. paratuberculosis* in the milk and the organism has been isolated from semen of infected bulls. Although the disease may be transmitted to young calves through infected milk, venereal transmission is not considered important. The predilection site for the organism is the intestinal tract where it stimulates a cell mediated hypersensitivity reaction resulting in the development of typical lesions with thickening of the intestinal wall due to infiltration of macrophages, giant cells, plasma cell and lymphocytes. The time taken for the development of lesions vary considerably from 15 months to several years.

The thickening of the intestinal mucosae causes distortion of the enterocytes resulting in a protein losing enteropathy. Although there is hepatic protein synthesis hypoalbuminaemia occurs.

18.14.5 Findings

Since Johne's disease has a long incubation period, it is almost always seen in adult cows and bulls between 3 and 6 years of age. Development of the clinical disease often follows a period of stress such as calving or around peak lactation. Initially, diarrhoea tends to be intermittent accompanied by a slight loss of weight and a drop in milk production. Gradually the diarrhoea become persistent and the drop in the milk yield is more dramatic. There is a also a loss of muscle mass especially around the hindquarters. There also may be transient submandibular oedema and intermittent pyrexia. Despite these clinical changes, the effected animals remain bright and continue to eat well. This may continue for several months but eventually if the

animals is not slaughtered it will become quite ill, anorexic and very dull. The disease runs a long course and terminates in death

18.14.6 Necropsy findings

The carcass in Johne's disease is highly emaciated. The lesions are confined to the terminal part of the small intestine and extending into the large intestine. In severe cases the entire intestine from the abomasum to the rectum may be affected. Macroscopically, the mucosa is thickened and corrugated and has a characteristic "morocco leather" like appearance. Histological section reveal infiltration of macrophages, giant cells, plasma cell and lymphocytes. There is shortening of the intestinal villi affecting absorption. Histological sections stained with Ziehl-Neelsen method will reveal acid fast organisms within macrophages and giant cells.

18.14.7 Diagnosis

The characteristic clinical signs, necropsy findings and histological examination of intestinal sections help in the diagnosis of Johne's disease. The organism may be demonstrated by microscopic examination of faeces or rectal mucosa, but several examinations need to be carried out before a definite diagnosis can be made. The presence of clumps of acid fast organisms would provide useful confirmation in living animals. The finding of single acid-fast organisms on microscopic examination is of doubtful significance as these are found in many apparently normal animals.

Faecal culture of *M. paratuberculosis* is possible but it takes at least 3 months on special media. An *in vivo* cell mediated-immunity reaction using the antigen, johnin PPD prepared from the organism offers a useful test. In a sensitised animal, a localised cellular response occurs with an increase in skin thickness at the site of injection, the test being read after 48 hours. A high percentage of infected animals react to the test but unfortunately negative cases (up to 50%) react to the test making it invalid. Serological tests available for laboratory diagnosis of Johne's disease include the complement fixation test and the fluorescent antibody test.

18.14.8 Treatment and control

Treatment is not effective even with drugs which have a high *in vitro* activity against *M. paratuberculosis*. Infected animals should be segregated and faeces properly disposed of. In endemic areas, vaccination of young animals with a vaccine in which live bacilli are suspended in lanolin is advisable, but a major complication of vaccination is that vaccinated animals become positive to both the Johnin and the tuberculin tests.

Further reading

Booksby, J.B. (1973) Observations on foot-and-mouth disease in Sri Lanka. *Ceylon Veterinary Journal.* **21**, 40-45.

Collin, C.H. and Grange, J.M. (1983) A review – the bovine tubercle bacillus. *Journal of Applied Bacteriology*. **55**, 13-29.

De Alwis, M.C.L. (1992) Haemorrhagic septicaemia - A general review. *British Veterinary Journal*. **148**, 99-112.

De Alwis, M.C.L. (1995) Haemorrhagic septicaemia (*Pasteurella multocida* serotype B:2 and E:2 infection) in cattle and buffaloes In: *Haemophilus, Actinobacillus and Pasteurella*. (Eds. Donachie, W, Lainson, F.A. and Hodgson, J.C.) Plenum Press. New York, USA pp. 9-24.

Faine, S. (1993) *Leptospira and Leptospirosis*. CRC Press, Boca Raton, Florida, USA.

Gilmour, N.J.L. (1976) The pathogenesis, diagnosis and control of Johne's disease. *Veterinary Record*. **99**, 433.

Hall, G.A., Jones, P.W. and Morgan, J.H. (1992) Calf diarrhoea. In: *Bovine Medicine*. (Edited by A.H. Andrew, R.W. Blowey., H. Boyd and R.G. Eddy), Blackwell Scientific Publication, UK. pp.154-180.

Mahamooth, T.M.Z. (1953) The saga of control and eradication of rinderpest in Ceylon. *Ceylon Veterinary Journal*. **1**, 73-78.

Sard, D. M. (1987) Clinical aspects of foot-and-mouth disease. *Veterinary Record* **102**, 186-187.

Scott, G.R. (1967) *Diagnosis of Rinderpest*. F.A.O., Rome.

St. George, T.D. (1988) Bovine ephemeral fever: A review. *Tropical Animal Health and Production*. **20**, 194-202.

Wijewardana, T.G.. Wijewardana, B.D.R., Appuhamy, W.N.D.G.S. and Premaratne, K.R.V.P.M. (1996) Prevalence of leptospiral antibodies in buffaloes in Sri Lanka. In: *The Role of the Buffalo in Rural Development in Asia*. [Edited by Perera *et al.*] SAREC/NARESA Buffalo Research and Development Project, Peradeniya, NARESA Press, Colombo SL. pp.415-426.

CHAPTER 19

Parasitic Infections in Cattle and Buffaloes

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Introduction

The control of parasitic infections is essential both for animal welfare and for optimising production. Some infections should be controlled for public health reasons, for example, if there is zoonotic potential or causes for condemnation of offal or carcasses at the slaughterhouse. Parasitic infections are caused by helminths, protozoa and arthropods. Thus, they are described in this chapter under the following headings

Helminths - Nematodes
Cestodes
Trematodes
Protozoan
Arthropodes

19.1 Helminth infections

19.2.1 Nematodiasis

19.2.1.1 *Toxocariasis*

(a) *Species – Toxocara vitulorum*

This is most common in buffalo calves. However, cattle calves also can be affected. They are large round worms with blunt ends, up to 30cm long and 5mm in diameter. The anterior end is characterised by three lips. The patent infection is found almost exclusively in calves from the age of one month to three months and the major source of infection of calves is through colostrum and milk of the dam during first ten days of lactation.

(b) *Symptoms* - Symptoms are not specific. They are intermittent diarrhoea, steatorrhoea and colic due to intestinal obstruction, followed by weight loss and death.

(c) *Diagnosis* - Eggs with thick, pitted shells and can be detected in the faeces of calves. Sometime in adults, almost transparent worms appear in the faeces.

(d) *Treatment and Control* - Most of the anthelmintics are effective against *Toxocara vitulorum* in calves. One dose of treatment with Pyrantel pamoate 250mg irrespective of bodyweight is adequate between day 10 and day 15 after birth, in order to destroy all immature worms, that are acquired from milk.

19.2.1.2 *Trichostrongyle infections*

(a) Species - Haemonchus contortus, Oesophagustomum spp, *Trichostrongylus axei, Trichostrongylus coloubriformis, Cooperia* spp, *Bunostomum* spp, *Trichuris* spp.

Gastrointestinal nematodes are the cause of one of the common parasitic infections in ruminants. Usually two types of infections are seen. Acute infection is common in young animals particularly during the first turnout for grazing. The clinical manifestations are mainly diarrhoea, dehydration and sudden death. Chronic infections are most common and are asymptomatic but production losses are inevitable. Death is sporadic. Anaemia is commonly seen in blood sucking nematode infections such as *Haemonchus*, *Mecistocirrus* and *Bunostomum* infections.

(b) Epidemiology - Gastrointestinal nematodiasis is common during rainy seasons. Therefore, the infection is seasonal in the dry zone and the pattern of infection reflects the rainfall pattern of the area. However, it is also very common in wet zone.

(c) Diagnosis - Diagnosis is mainly based on the demonstration of nematode eggs in faeces by salt flotation. Faecal culturing and species identification based on morphology of infective larvae are useful for a definitive diagnosis. The intensity of infection can be estimated by McMaster egg count estimation.

(d) Control - Thus can be affected by strategic treatment with effective anthelmintics. The use of an effective anthelmintic prophylactically, just prior to the onset of rains and another administration after an interval of 3-5 weeks, would effectively minimise accumulation of infective larvae in the pasture and the subsequent reinfection potential.

19.2.1.3 *Lungworm infection:*

(a) *Species - Dictyocaulus viviparous*

Lungworm infection is not very common in Sri Lanka, but sporadic cases can be seen in the wet zone particularly in the upcountry. Parasitic pneumonia is a consequence of chronic infection and is evident in young calves.

(b) *Clinical Symptoms* - Mainly pneumonic signs can be seen.

(c) *Diagnosis* - Fully developed *Dictyocaulus viviparous* eggs can be detected in faeces as well as in nasal secretions which can be isolated by salt floatation method.

(d) *Control* - Control is not attempted in Sri Lanka since the disease is not endemic. Immunisation of calves using an irradiated larval vaccine has become a common method of control of the disease in endemic areas of the world . The vaccination schedule comprises two doses of approximately 1000 irradiated larvae administered four weeks apart and calves are not turned out for grazing until at least two weeks after administration of the last dose of vaccine.

19.2.1.4 *Filarial worm infections:*

(a) *Species - Setaria digitata, Setaria labiato-pappilosa* and *Onchocerca gibsoni*

Filarial worms are common in cattle and buffaloes in Sri Lanka particularly in the dry zone. About 80% of the cattle population and 52% of the buffalo population are effected.

Setaria spp. usually are found in the peritoneal cavity of infected animals and are asymtomatic carriers of microfilaria. However, *Setaria* infection causes considerable economical losses in small ruminants, causing paralysis due to migration of infective larvae in the central nervous system

19.2.2 Cestodiasis

19.2.2.1 *Adult worm infection:*

(a) *Species - Moniezia expansa, Moniezia benedini* and *Aviteliina centripunctata*

These are common tapeworms infecting all domestic livestock in Sri Lanka. Adult *Moniezia* worms are up to 6m long and *Avitellina* are about 3 m long. The life cycle of *Moniezia* is indirect, involving many species of orbated mites as intermediate hosts. Ruminants are infected by ingestion of the infected mites with herbage.

(b) *Symptoms* - Generally, they are inapparent infections. Heavy infections are found only in young animals which can cause reduced weight gain. Masses of *Moniezia* can cause obstruction in the intestine.

(c) *Diagnosis* - Proglottids, which look like cooked rice grains, containing typical thick-shelled or imperfectly rounded eggs, appear in the faeces.

(d) *Treatment and control* - Niclosamide (75-90 mg/kg) and praziquentel (5mg/kg) are specific cestocidal drugs. In addition, some of the benzimidazoles are effective against tapeworms (Table 20.1). However, it is advisable to modify the treatment and control strategies selected for nematodes, to include the use of a broad spectrum anthelminthic that controls both tapeworms and roundworms. A number of the benzimidazoles, such as albendazole, cambendazole and mebendazole have proven efficient in the control of tapeworms.

Control measures may also be directed towards the intermediate host, the orbated mites. It has been shown that the habitat of the mites can be destroyed by ploughing and where this is feasible, new sown pastures can be kept free of tapeworms for several years.

19.2.2.2 *Intermediate stages of tapeworms*:

(a) Hydatidiosis

(i) *Species - Echinococcus granulosus*

(ii) Location - Larvae (Hydatid cysts) of *E. granulosus* are found in the liver and lungs of cattle, sheep, goats and other domestic animals. Adult worms are found in the small intestine of the dog and other related carnivores.

(iii) *Symptoms* - Hydatid cysts do not usually cause clinical signs and symptoms unless the cysts are numerous or become very large.

(iv) *Significance* - Hydatidosis in cattle and goats is not a serious problem, but infected organs are condemned at slaughter. In man, hydatidosis is a serious problem but has not been reported in Sri Lanka.

(v) *Diagnosis* - Usually the diagnosis of hydatidosis is made at slaughter or necropsy.

(vi) *Control* - Control of hydatidosis is therefore based on prophylaxis of the infection in man, livestock and dogs. All abattoirs, slaughterhouses and slaughter slabs should rigorously keep dogs from gaining access to the premises and all offal and condemned material containing hydatid cysts should be destroyed.

Several anthelminthics are available for treatment of dogs, but a number of these cause the segments to disintegrate, allowing the eggs to remain viable and thus spread the infection. If it is possible, faeces that are passed after treatment should be buried or burned. However, experience gained during several control programs has shown that eggs are of minor importance in the epidemiology of hydatidosis. An alternative treatment is to use the purgative arecoline hydrobromide which is given to dogs following 12 hours of fasting. The parasites will be expelled during the subsequent 6 hours during which time the animal should be confined and the faeces should be destroyed.

Public education programs should convey the message that dogs infected with *E. granulosus* present a danger to the human population and livestock. Farmers and dog owners should know that (a) uncooked viscera containing hydatid cysts should not be fed to dogs, (b) dogs should be dewormed regularly and (c) handling of infected dogs may increase the risk of infection.

(b) Muscular Cysticercosis

(i) *Species - T. saginata*

(ii) *Location* - Bovine cysticercosis is caused by the presence of the vesicular larvae *Cysticercus bovis* in the striated muscles of cattle. Cattle become infected when they ingest eggs of *T. saginata*. Poor standards of personal hygiene of infected human populations are responsible for the spread of cysticercosis. Abnormal eating habits of cattle due to certain

mineral deficiencies (pica) may result in a craving that increases exposure through the ingestion of faeces.

(iii) *Symptoms* - Cysticercosis do not usually cause clinical signs and symptoms.

(iv) *Significance* - During meat inspection infected carcasses should be condemned.

(v) *Diagnosis* - The cysticercus appears as a small (6-10 mm) oval vesicle which is at first semitransparent. These lesions are known as beef measles (in myocardium, tongue, masseters and shoulder muscles).

(c) Abdominal cysticercosis

(i) *Species* - Abdominal cysticercosis of ruminants is caused by *Cysticercus tenuicollis*, the larval stage of *T. hydatigena*, a dog tapeworm.

(ii) *Location* - Migratory post-oncospheral stages may occasionally be found in the liver parenchyma and thin-necked cysts are often found attached to the omentum, the intestinal mesentery and to the serosal surface of abdominal organs.

(iii) *Significance* - The developed *cysticercus tenuicollis* has no pathogenic affect while in the abdominal cavity. When many embryos migrate simultaneously through the liver, clinical signs may be seen. The migration may causes severe destruction of liver tissue and the pathology seen in the liver may be similar to that observed in liver fluke infections.

(iv) *Diagnosis* - The fully developed cyst is a large (5 cm or more in diameter), soft, semitransparent bladder with the invaginated head of the tapeworm clearly visible.

(v) *Control of Cysticercosis* - At present it is not considered feasible to treat animals due to the high cost of drugs and the possible public health significance of dead, calcified cysts in meat and organs. Drugs, which have shown effect against larval cestodes, include praziquantel, mebendazole and albendazole. Control of cysticercosis and the adult tapeworms is therefore based on the prophylaxis of the infections. To prevent infection of cattle, sheep and goats, all *Taenia* final hosts namely man (*Taenia saginata*) and dogs (*Taenia hydatigena*) should be treated. In addition, humans should be educated on the importance of maintaining high standards of personal hygiene in order to prevent the spread of *T. saginata* eggs.

Standardized methods of meat inspection should be implemented to detect the infection at slaughter, and infected meat and organs should be condemned or treated (cooked until grey, or frozen) to prevent infected meat reaching humans and dogs.

19.2.3 Trematodiasis

19.2.3.1 *Rumen fluke infections:*

(a) Amphistomes (Species of Paramphistomidae)

(b) *Location* - Adult parasites are found in the rumen and immature flukes in the small intestine. A large number of species of this family has been described. Water snails are involved in the development of the parasite through an indirect life cycle. Infection is very common in the dry zone. The cercariae encyst on herbage that is in contact with water and develop into the infective stages (metacercaria). Infection is acquired by ingestion of herbage contaminated with metacercaria, especially around water holes. The adult flukes are generally non-pathogenic. Disease occurs when masses of immature worms attach themselves to the intestinal mucosa after encystment causing tissue destruction and inflammation. The infection peaks usually at the end of the dry season. Troughs with standing water and other water bodies are preferred habitats of the intermediate hosts.

(c) *Symptoms* - Enteritis with extensive diarrhoea, hypoproteinaemia, weakness are evident during the intestinal phase, when immature worms irritate the small intestinal mucosa. Symptoms are most evident mainly in young stock. The infection with adult worms is generally inapparent.

(d) *Diagnosis* - This is based on the demonstration of immature flukes in the diarrhoeic faeces and the history of previous cases in the area.

(e) *Treatment* - Niclosamide (90mg/kg bwt), resorantel (65 mg/kg bwt)

(f) *Prophylaxis* - Infection could be avoided by supplying herds with clean water particularly during the dry season.

19.2.3.2 *Liver fluke infections:*

(a) *Species - Explanatum explanatum* (amphistomes)

This is a very common amphistome in buffaloes and usually 80% of the buffaloes are effected.

(b) Location - Adult parasites are found in the bile duct of the liver and immature flukes in the small intestine. The life cycle and behaviour of parasites is very similar to that of rumen amphistomes.

(c) Symptoms - The immature stage in the small intestine causes enteritis as with ruminal amphistomes. Infection with adult worms is generally non-pathogenic, but heavy infection causes emaciation, weakness and loss of appetite. The main significance of the infection is that it causes economic losses due to condemnation of the liver during meat inspection.

(d) Diagnosis - This is based on the demonstration of immature flukes in the diarrhoeic faeces and on reports of previous cases in the area.

(e) Treatment - Niclosamide (90mg/kg bwt), resorantel (65 mg/kg bwt)

(f) Prophylaxis - Infection can be avoided by supplying the herds with clean water, and preventing grazing around water sources particularly during the dry season.

19.2.3.3 *Nasal fluke infections:*

(a) Species - Schistosoma nasalis: Parasites occur in the veins of the nasal mucosa in buffaloes and cattle. Infection is more prevalent in buffaloes than in cattle, but clinically it mainly affects cattle, particularly young animals.

(b) Symptoms - Parasitized adult worms and eggs of female worms produce granuloma in the nasal cavity particularly the mid region. Affected animals show difficulty in breathing, nasal discharge and snoring while breathing.

(c) Diagnosis - Characteristic eggs (Boomerang appearance) can be demonstrated in the faeces and nasal discharge of infected animals.

19.2.3.4 *Blood fluke infections:*

(a) Species - Schistosoma spindale – This parasite occur in the mesenteric veins of

Table 19.1 Drugs effective against common helminths infections in ruminants

Parasites	Anthelmintics	Dosage (mg/kg) cattle	Goats/Sheep
Gastro-Intestinal Nematodes	Albendazole 7.5	5(PO)	
	Fenbendazole	7.5	5(PO)
	Levamisole	7.5	7.5(PO)
	Oxibendazole	7.5	5(PO)
	Febental 7.5	5	
	Ivermectin	0.2	0.2(PO or SC)
Lung worms	levamisol 7.5	7.5(PO or SC)	
	Fenbendazole	7.5	5(PO)
	Albendazole	7.5	5(PO)
	Ivermectin	0.2	0.2(PO or SC)
Tape worm infection	Oxyclozanide	10	15
	Praziquantel	10	10(PO)
Trematode infection	Closantel 10	10(PO)	
	Oxyclozanide	10	15(PO)
	Rafoxanide	7.5	7.5(PO)
	Triclabendazole	12	10(PO)

PO - per oral
SC - Subcutaneously

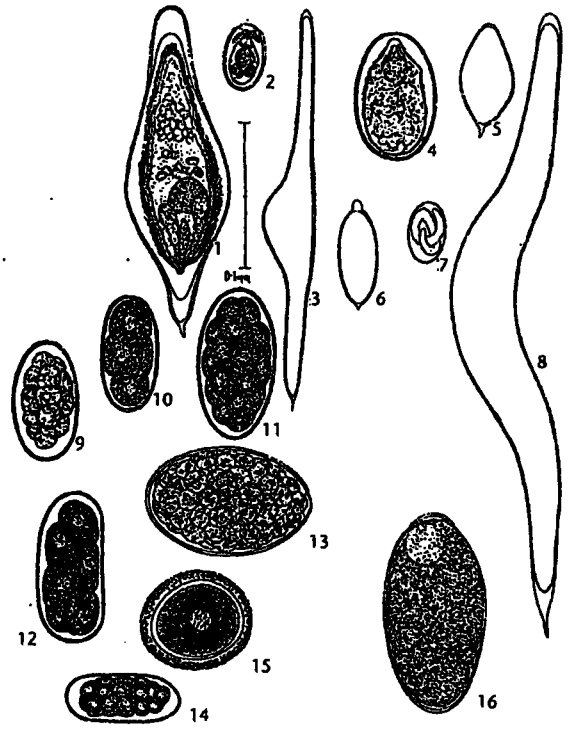

Fig. 31 Eggs of helminth parasites of cattle (not drawn completely to scale) [3]
(1) *Schistosoma bovis*, (2) *Eurytrema pancreaticum*, (3) *Schistosoma spindale*, (4) *Schistosoma japonicum*, (5) *Schistosoma indicum*, (6) *Ornithobilharzia turkestanicum*, (7) *Thelazia rhodesi*, (8) *Schistosoma nasalis*, (9) *Oesophagostomum radiatum**, (10) *Mammomonogamus laryngeus*, (11) *Mecistocirrus digitatus*, (12) *Bunostomum phlebotomum**, (13) *Carmyerius spatiosus*, (14) *Cooperia pectinata**, (15) *Toxocara vitulorum* and (16) *Fasciola hepatica*.
* Strongyle-type eggs are difficult to differentiate in routine examinations

Fig. 19.1 Eggs of worm parasites of cattle and buffaloes.

cattle and buffaloes. The major clinical signs associated in the intestinal and hepatic forms (that develop after the onset of the egg excretion) consist of enteritis, intestinal atony, anaemia and progressive emaciation in heavily infected animals.

(b) Diagnosis - Spindle shaped eggs in the faeces are evident

(c) Treatment - Treatment of schistosomiasis is not easy. Highly efficient anthelmintics may have fatal consequences because dead worms may cause massive thrombus formation in the liver. Therapy should be aimed at killing the worms over a long period of time to avoid the risk of massive damage that can be caused by the simultaneous death of all worms. Praziquentel at 25mg/kg body weight is effective, and two treatments, 3-5 weeks apart are required.

19.3 Protozoan infections

19.3.1 Bovine coccidiosis

(a) *Species - Eimeria zuernii, Eimeria bovis, Eimeria ellipsoidalis, Eimeria baroillyi, Eimeria subspherica, Eimeria cylindrica, Eimeria auburnensis.* In buffalo calves *Eimeria baroillyi* seems to be the most prevalent *Eimeria,* associated with diarrhoea.

(b) *Symptoms* - Coccidiosis is a common disease of young cattle and occurs where standards of hygiene is low. Haemorrhagic or viscous diarrhoea, dehydration, weight loss are common signs and death can occur rapidly mainly in calves. The animals that survive may remain permanently stunted.

(c) Diagnosis - Clinical signs and the presence extremely high numbers of oocysts per gram of faeces.

(d) Therapy - The drugs commonly used to treat clinical coccidiosis include sulphonamides, nitrofurazone, amprolium (10 mg/kg po.>10 days) and monensin (1 mg/kg per day po. for up to 6 weeks). Sulphadimidine (33% solution for intravenous injection); Sulphamethazine (50-100 mg/kg, po. daily for 4 days) and sulphaquinoxaline (15 mg/kg, po. daily for 3 days) may be used to treat sick animals. Sulphaguanidine is less effective than the soluble sulphonamides, which can be administered orally or parenterally. Sulphonamides, combined with trimethoprime can also be used to treat clinical coccidiosis while Toltrazuril (1x20 mg/kg per day) is highly effective in ruminants.

(e) *Control* - Calves should be kept in clean dry quarters and watering devices should be protected from faecal contamination. Decoquinate (0.5 mg/kg per day in feed for at least for 28 days), amprolium (5-10 mg/kg daily for 21 days) and ionophorous antibiotics (e.g. Monensin, 1 mg/kg daily for 30 days) would prevent the clinical entity in calves. Neonates should receive colostrum within 6 hours after birth and stress factors (e.g. weaning, sudden change in feed, etc.) should be minimized.

19.3.2 Bovine cryptosporidiosis

(a) *Species - Cryptosporidium parvum*

(b) *Symptoms* - Especially calves younger than 8 weeks may be affected, showing different degrees of diarrhoea, associated with oedema and hyperplasia of the mesenteric lymph nodes that does not respond to therapy. *Cryptosporidium parvum* is pathogenic to calves, sheep, pig, rat and man. Bovine cryptosporidiosis is a zoonotic disease. Combinations of the infection with rota and/or corona viruses are common and result in persistent diarrhoea, emaciation and death.

(c) *Diagnosis* - Spherical oocysts 4.5-5 mm in diameter are passed in the faeces. Oocysts may be demonstrated in Ziehl-Neelsen's carbol-fuchsin stained smears of diarrhoeic faeces.

(d) *Therapy* - No effective specific treatment is known. Supportive therapy, such as rehydration and antibiotics may help in mild cases. Chemoprophylaxis of bovine cryptosporidiosis can be achieved experimentally with halofuginone lactate (60-120 mg/kg/day, po. for 7 days) or paromomycin (100 mg/kg/day for 11 days).

(e) *Control* - Animals with a massive oocyst excretion should be separated from other animals and man. Contamination with faeces should be avoided. Oocysts are resistant to many disinfectants (exception, 5% formalin). Emphasis should therefore be on regular removal of contaminated faecal material.

19.3.3 Babesiosis

(a) *Species* - *B. bigemina* and *B. bovis* infections are the most important causes of disease of *Bos taurus* cattle and their crosses in the hill country and mid country areas of Sri Lanka. The disease is transmitted by the vector, *Boophilus spp.*. The disease caused by *B. bovis* is similar to that caused by *B.bigemina*. Affected animals may die depending on the virulence of the organism. In some regions *B.bovis* is more virulent than *B.bigemina*. Mortality rates are very high (30% for *B. bigemina* and 70-80% for *B. bovis*).

(b) *Symptoms* - Infections with *B. bigemina* are characterized by a haemolytic syndrome, including continuous fever (40-41°C), high parasitaemia (2.5-10%), anaemia, icterus (intense yellow-brownish ocular, gingival and vaginal mucosae) and haemoglobinuria (dark brown, frothy urine) and general depression. Abortion and agalactia in dairy cows are early signs. The symptoms are less pronounced in the subacute form. *B.bovis* infections are dominated by a shock syndrome, leading to death following a steep rise in temperature in the peracute form. The acute disease is accompanied by fever, ataxia, pedaling movements and overt aggressivity (attacks). Haemolysis, icterus and haemoglobinuria are less apparent than in other babesioses.

(c) *Diagnosis* - Clinical diagnosis is difficult and can be confused with leptospirosis (peracute disease) or anaplasmosis (chronic in nature). Splenomegaly, icterus and haemoglobinuria and thickened bile in the gall bladder are characteristics of haemolytic babesiosis. Petechiae and microinfarcts suggest infections with *B.bovis*. The presence of *Babesia* parasites in blood smears of animals without clinical symptoms does not necessarily support the diagnosis of babesiosis. This can be the result of a resurgence of a chronic infection following an immunosuppression due to another disease. In *B. bigemina* infections, the severity of the disease can be deduced from the percentage of parasitized red blood cells (<0.1% mild, 0.5-1% subacute, 5-10% severe infection), whereas in *B.bovis* infections the presence of parasites indicates babesiosis, because the parasite concentration in the peripheral blood is considerably lower than in the organs. In acute *B. bovis* infections impression smears of congested organs show punctiform babesial bodies in agglutinated erythrocytes.

(d) *Therapy* - Diminazene aceturate (3-5 mg/kg, im.), Quinuronium sulphate (1-2 mg/kg, sc.or im.), Imidocarb (1-3 mg/kg sc or im) and amicarbalide disethionate (5-10 mg/kg, im.) can be used against the pathogenic *Babesia* species of cattle. When treating animals two points should be considered: (1) For serious clinical attacks, the

dose should be split to avoid shock due to massive destruction of *Babesia*. (2) Most babesicidal drugs are toxic. In endemic unstable areas (hill country and mid country), it is preferable to support immunity within the population, as such animals can withstand repeated infections. The aim of therapy is therefore to reduce but not to eradicate the parasite concentration within the host. In severely affected animals emphasis should also be given to supportive treatment (electrolytes, rehydration, iron-dextran, etc.) and good nutrition during the convalescence.

(e) *Control* - Several methods are used in endemic areas to control or prevent babesiosis (i) Immunization with attenuated *B. bovis* and *B. bigemina* strains. Presently a vaccine is produced at the VRI using these two *Babesia* spp. which will be made available on request, (ii) Prophylaxis through tick control: Tick control is essential in endemic areas and should be practised once in 3 weeks during periods of increase tick activity.

19.3.4 Bovine Sarcosporidiosis

(a) *Species* - *Sarcocystis bovicanis* (syn. *S. cruzi*), *Sarcocystis bovifelis* (syn. *S.hirsuta) and Sarcocystis bovihominis* (syn. *S. hominis)*

(b) *Hosts* - Cattle are intermediate hosts of three *Sarcocystis* species while dogs (*S. bovicanis*) and cats (*S. bovihominis*) are final hosts.

(c) Species description - Cysts of several *Sarcocystis* species are found in the muscle tissues of cattle. Cattle always act as intermediate hosts. *S. bovicanis* is the most pathogenic species in cattle.

(d) *Symptoms* - Anorexia, intermittent fever, anaemia, weight loss have been observed after experimental infection in calves. Deaths occur about 30 days after a heavy infection with 10^5-10^6 sporocysts. Reduced milk yield, loss of condition, dyspnoea and abortion have been observed in adult cattle. Once muscle cysts are formed the infection is inapparent.

(e) *Diagnosis* - This is very difficult in the acute phase (1 month post infection). During this period schizonts may be demonstrated in vascular endothelial cells by means of stained-scrapings taken from the blood vessels or by histopathological examination. Necropsy might reveal the muscle cysts. Serological tests (IFAT, ELISA) are indicated after the acute phase. The PCR may be used as a highly sensitive method to detect *Sarcocystis* infections.

(f) *Therapy* - This is extremely difficult in cattle and rarely indicated. Amprolium (100 mg/kg, po., daily for 30 days) is effective to avoid clinical signs in cattle during experimental infections.

(g) *Control* - The contamination of cattle food with faeces of dogs, cats and man as well as the feeding of raw cattle meat to dogs should be avoided.

19.4 Arthropod infestations

19.4.1 Ectoparasites

The most important group of ectoparasites that inhabit cattle in Sri Lanka are the ticks. They are obligate parasites and are the most widespread of all ectoparasites. The commonest genus involved is *Boophilus*. The other important genera of ticks recorded are *Amblyomma, Haemophysalis, Hyalomma* and *Riphicephalus.*

Besides ticks, a variety of biting flies feed on ruminants. The most important of these are *Stomaxys calcitrans*, (stable fly), *Haematobia irritance* (horn fly), *Tabanus* and *Haematopota.* Their economic importance include transmission of *Trypanosoma evansi* and *Anaplasma* species and sucking of considerable amounts of blood. Myiasis causing genera such as *Lucillia, Musca* and *Caliphora* are also notable as ectoparasites in cattle.

Finally, lice also have been identified in cattle reared in the low country. The important species of lice in cattle include *Lingnathus vituli, Haematopinus eurysternus, H. quadripertusus.*

19.4.1.1 Diagnosis

Identification of ectoparasites is a specialized task which requires the assistance of an entomologist. Samples should be preserved and dispatched in 75% ethanol or 2-10% formalin. Temporary preservation can be achieved in industrial alcohol, medical methylated spirit or similar alcoholic liquids. The adults of large insects can be preserved dry, whereas mites and smaller insects such as lice could be preserved on microscope slides.

Adult flies visiting the host are usually caught by netting, using various traps or collected after being killed by insecticides. Larvae can be collected in sheds where animals are housed or directly from animals where the larval stages are parasitic.

The detection of small ectoparasites such as lice depends on close examination of the animals body surface. Eggs are also found attached to hair. Ticks may be collected off from the host with a piece of cotton wool soaked in anaesthetic placed around ticks. To recover ticks from pasture, drag a blanket over the ground to which the unfed ticks become attached, as they would do a host.

For demonstration of mites, it is often necessary to obtain a skin scraping at the edge of a visible lesion after the hair over this area has been clipped away. A portion could be examined in the field either with a hand lens against a dark background or by low power microscopy.

19.4.2.2 Control

Integrated strategies for the control of ectoparasites of veterinary importance are already being implemented for selected pest species in many parts of the world.

Within the next decade, the integration of technologies for the management of arthropod pests will become the standard practice rather than the exceptions. Simply stated, integrated pest management, (IPM), consists of the systematic application of two or more technologies, in an environmentally compatible and cost effective manner, to control arthropod pest populations which adversely affect livestock.

Chemical control (Table 19.2) will probably always be a component of IPM systems. The practical use of pesticides, whether traditional groups of compounds (e.g. organophosphates, carbamates, formamidines and synthetic pyrethroids) or newer groups of compounds (e.g. insect growth regulators or avermectin) forms an integral part of almost every IPM system. In a tick fever endemic area, care must be taken not to reduce tick numbers to low levels as this will result in the loss of resistance to tick fever. A few ticks are required to maintain transmission of the causal organism to immunize the calves and to maintain the resistance of older cattle. Acaricides could be used strategically. If cattle could be treated every 21 days, this will break the cycle because the lowest time from the time of dropping off an engorged female to the time that larvae are ready for reinfestation is 18 days and the treatment for protection is given for 3 days; so that the 21 day treatment regime will break the cycle. This should only be carried out during high tick activity during the rainy season.

Mechanical control can provide a simple and helpful addition to some IPM systems. For example, effective manure management and disposal can reduce fly populations by as much as 50% in cattle and buffalo farms. Trapping also has a potential role in reducing fly populations in the vicinity of stock. This technology relies on dependable

Table 19.2 Some common compounds used for the control of ectoparasites of ruminants

Compound	Pest
Organophosphates	
Chlorpyrifos	Lice, horn flies
Coumaphos	Lice, horn flies, ticks, mites
Crotoxyphos and dichlorvous	Lice, horn flies, house flies, stable flies, ticks, mites
Dichlorvous	horn flies, house flies, stable flies
Diazinon Trichlorphon (metrifonat)	Lice, ticks, mites
Carbamates	
Carbaryl	Lice, ticks, mites, flies
Promocyl	Lice, ticks, mites, flies
Chlorinated Hydrocarbons	
Lindane	Lice, ticks, mites, flies
Methoxychlor	Lice, ticks, mites, flies
Toxophen	Lice, ticks, mites, flies
Pyrethrins and Pyrethroides	
Cypermethrin	Horn and face flies, ticks
Flumethrin	Flies, ticks
Permethrin	Lice, horn flies, face flies, ticks, mites
Pyrethrin	Lice, horn flies, ticks
Avermectins	
Doramectin	Mites, lice, ticks
Ivermectin	Mites, lice

attractants, functional trap designs, proper trap placement and regular servicing. In the case of integrated tick management systems, mechanical control components have included vegetation management as well as wildlife host management.

Ectoparasite management through regulatory control will also be an important component of future IPM systems. This approach is particularly relevant to programmes implemented over broad geographical areas by government agencies. The establishment of quarantine regulations and procedures is certainly of enormous value in limiting the distribution of those ectoparasites, which depend on livestock movements. Surveillance is another important regulatory technique when the problem reaches unmanageable proportions. Regulatory control is indispensable when dealing with indigenous arthropod pests.

The potential for multiplication is enormous, since each female adult can lay several hundred eggs over a few days and given the right conditions, each egg can become another adult in less than two weeks. Eggs of flies can undergo development under in certain conditions. The moisture level must be in the range of 50-70% of the content of fresh layer manure and all development is temperature dependent. Thus where manure dries quickly as a result of the good ventilation or where a large volume of water is added, fly breeding may be limited. Conversely, where water is accidentally added to relatively the dry manure mass, as may happen with leaking water systems, the potential for fly breeding is increased. It is better to limit manure removal during the fly breeding season. This allows equilibrium to be established between flies and their predators.

Further reading

Faizal, A.C.M., Rajapakse, R.P.V.J., Horadagoda, N.U. and Paranagama, W. D., (1996) Cost-effectiveness of Pyrantal Pamoate as an anthelmintic against gastro-intestinal nematodes of goats. (Abstract) *The Sri Lanka Veterinary Journal*, **44**, 19.

Faizal, A.C.M., Rajapakse, R.P.V.J., Jayasinghe, S.R. and Rupasinghe, V. (1999) Prevalence of Eimeria spp and gastrointestinal nematodes versus weight gains in treated goats raised in the dry areas of Sri Lanka. *Small Ruminant Research*, **1806**, 1-5.

Noordeen, F., Rajapakse, R.P.V.J., Faizal, A.C.M. and Horadagoda, N.U. (1999) Preliminary investigations on the occurrence of Cryptosporidium infection among goats in three climatic zones of Sri Lanka. In: *Proceedings of Fifty First Annual Sessions of Sri Lanka Veterinary Association.* p.73.

Paranagama, W. D., Rajapakse, R.P.V.J., Faizal, A.C.M. and Horadagoda, N.U. (1996) A preliminary study on an epidemiological study on gastrointestinal nematodiosis in goats managed under village conditions in dry zone. Paper was presented at the forty ninth of Annual Scientific Sessions of Sri Lanka Veterinary Association, 7th - 8th March 1997. (Abstract) *Sri Lanka Veterinary Journal*, **44**, 29

Paranagama, W. D., Rajapakse, R.P.V.J., Horadagoda, N.U. and Faizal, A.C.M. (1997) Correlation between naturally occurring caprine *Haemonchus contortus* infection and the packed cell volume, haemoglobin concentration, total protein. *Tropical Agricultural Research.* **9**, 299-235.

Rajapakse, R.P.V.J., Lloyd, S. and Fernando, S.T. (1994) *Toxocara vitulorum*: Maternal transfer antibodies from buffalo cows *(Bubalis bubalis)* to calves and levels of infection with *T. vitulorum* in calves. *Research in Veterinary Science.* **57**, 81-87.

Rajapakse, R.P.V.J., Faizal, A.M.C. and Horadagoda, N.U.. (1996) Efficacy of Albendazole and Levamisole against gastrointestinal nematodes in goats in Sri Lanka: A preliminary investigation. In: *Proceedings of Fifty Second Annual Sessions of Sri Lanka Association for the Advancement of Science.* p. 5 (ISSN 1391-023X).

Seneviratne, P. (1967) Economic losses due to parasitic diseases of livestock in Ceylon. *Ceylon Vet. Journal.* **15**, 5-8.

CHAPTER 20

Metabolic Disorders in Cattle and Buffaloes

L. N. A. de Silva

Metabolic disorders may occur as a result of the derailment of metabolism of minerals, energy and also changes in management practices in livestock. The most commonly encountered metabolic disorders, particularly among dairy cattle and buffaloes are hypocalcaemia (milk fever), hypomagnesaemia and acetonaemia (ketosis).

20.1 Hypocalcaemia (Milk fever)

This condition arises as a result of a metabolic disorder that occurs at or around the time of parturition in mature dairy cows. It is characterised by general muscular weakness, hypoaesthesia and circulatory collapse.

20.1.1 Aetiology

In pregnant cows and heifers, the concentration of ionised calcium in the plasma declines at or just after calving. When this decline is excessive, parturient paresis develops. The normal plasma concentrations of calcium, phosphorus and magnesium are between 2.0-3.0 mmol/l, 1.1-2.8 mmol/l and 0.7-1.0 mmol/l, respectively. Most animals with clinical signs suggestive of hypocalcaemia show a low Ca^{2+} concentration in serum (< 2.0 mmol/l). In a large number of cases of milk fever, the mean serum phosphate concentration is found to be 1.2 mmol/l, while there can be a low or high magnesium concentration in serum.

Only about 50 per cent (approximately 8g) of the calcium in the blood is ionised, which is immediately available. This ionised calcium is usually called the "calcium pool". Calcium goes into this pool from the food (approx. 1 g/hr) and the bones (approx. 1 g/hr) and at the same time, calcium is also lost by excretion (approx. 1 g/hr) and absorbed into the bones (approx. 1 g/hr). During gestation, there is increased absorption from the gut. At parturition, there is a sudden increase in the demand for available calcium, of about approximately 1 g/hr for incorporation into colostrum/milk that is produced. The ionised fraction of calcium is derived either from dietary or skeletal sources. It is when the decline in calcium level is excessive and the calcium concentration in blood falls below 2.0 mmol/l, that clinical signs of parturient paresis become apparent.

On the first day of lactation, the demand for calcium increases to about three times the total available calcium in the pool (i.e. approximately 24 g compared to 8 g). However, it takes about two days for the calcium homoeostatic mechanism to respond to this sudden demand associated with the onset of lactation. The efficiency of calcium absorption from the gut increases as soon as milk production begins, whereas it may take up to two weeks for significant quantities of bone calcium to be mobilised.

The failure of a cow immediately after calving to mobilise sufficient calcium to meet this sudden increase in demand results from the interaction of a number of factors, many of which are as yet not full understood.

At around the time of parturition, there is stasis of the alimentary tract for a short period and as a result the animal's appetite decreases. While the food intake of heifers drops only slightly, that of mature cows may decrease to about 20 per cent of her preparturient intake. This period of reduced calcium intake, which can persist from 8 to 20 hours, is crucial in determining the degree of hypocalcaemia because absorption from the gut constitutes the dominant source of calcium to the pool. Furthermore, as the cow gets older, the amount of calcium which can be mobilised quicky from the bones decreases progressively. This means that there is an increased dependence on the diet as the major source of calcium and this makes the individual increasingly susceptible to hypocalcaemia particularly when the food intake is suddenly decreased.

Calcium homeostasis depends on a balance between the levels of parathyroid hormone (PTH) which is secreted by the parathyroid gland and calcitonins which are secreted by the thyroid gland. Parathyroid hormone is secreted in response to decreasing plasma concentrations of calcium. This results in resorption of calcium from the bone leading a slow rise in plasma calcium concentration. At the beginning of lactation, there is no failure in PTH secretion, but it would appear that the target organ (bone) is non -responsive. This may be due to (a) a deficiency of its specific primary target, adenyl cyclase enzyme, (b) a deficiency of vitamin D or (c) the presence of PTH antagonists such as calcitonins, cortisol and oestrogen. Calcitonin is secreted in response to increasing plasma calcium concentrations. It inhibits bone resorption and can quickly lower the concentration of calcium and phosphorus in the plasma. If the diet is high in calcium, calcitonin secretion will also be high, bone resorption will be minimal and consequently the hypocalcaemic state at parturition is exacerbated. When the thyroids in milk fever cases are examined, they appear to be depleted of calcitonins, thus confirming the theory that excess calcium is being absorbed and the plasma concentration is down to normal levels, by inhibiting PTH-stimulated resorption of Ca++ from the bone.

20.1.2 Epidemiology

Milk fever is a modern disease and almost invariably affects high yielding dairy cows. Recent evidence suggests that all the dairy breeds have a similar susceptibility although it has long been accepted that the Jersey breed is much more susceptible than the other dairy breeds. However, there is no doubt that within breeds, the condition occurs regularly in certain families. This disorder is rare in heifers, uncommon at second calving, the highest incidence being in 5 to 6 year old cows. The incidence is highest in the hill and mid-country areas of Sri Lanka and clinically detectable cases are found throughout the year. About 10 per cent of the cases occur during the two days prepartum, while 80 per cent occur in the first three days postpartum (usually 12-36 hours) and the remainder (10%) after three days. There are reports of hypocalcemia in high yielding dairy buffaloes. However, the occurrence of this condition in buffaloes is much less in Sri Lanka.

20.1.3 Clinical Signs

A cow with milk fever can progress to death through three clinically recognisable stages.

20.1.3.1 *Prodromal Stage*: Cows tend to be apprehensive, tending to paddle especially with the hind legs. There is wide spread muscle tremor, slight hyperaesthesia and smooth muscle paralysis, which results in an inability to swallow. Consequently, anorexia and adipsia is evident followed by ruminal stasis, with the passing of a small amount of dry faeces or no faeces and the suspension of micturition. These signs can regress spontaneously or they can become more severe. At this stage, the cow may fall down and after attempting to get on to her feet several times, she may continue to lie in sternal recumbency.

20.1.3.2 *Sternal recumbency*: Once an individual has become recumbent, spontaneous recovery is very unlikely. The cow becomes increasingly hypoaesthetic and tends to lie with her head tucked into her flank. The temperature is subnormal, muzzle is dry and, because of ruminal stasis, ruminal tympany becomes obvious. The degree of hypoaesthesia gradually becomes worse and eventually the cow goes into lateral recumbency.

20.1.3.3 *Lateral recumbency*: The respiration rate decreases (10/minutes) and groaning respirations may be heard. The heart rate increases but the heart sounds become increasingly more difficult to hear. The pupillary reflex is absent and the pupils become more and more dilated. There is a worsening of the above signs and the effected animal may eventually succumb to the disease condition.

In the few cases which occur prepartum, there is the added complication of uterine inertia. Therefore, in such cases where this condition develops immediately after parturition, it is advisable to check whether there is another calf in the uterus. Other complications include uterine prolapse, retained placenta and aspiration pneumonia. It has been estimated that milk fever results in a reduction of 3 to 4 years in the potential productive life of a dairy cow and that once an individual has had the disease, there is a greater chance of a recurrence.

20.1.4 Diagnosis

Milk fever should be suspected when paresis occurs around parturition in a mature dairy cow. Other conditions which should be considered include injuries associated with parturition (nerve paralysis, muscle rupture, fracture/ dislocations) or infection (peritonitis, acute coliform mastitis). There are no specific macroscopic or microscopic lesions typical of parturient paresis at post mortem.

20.1.5 Treatment

Any animal suspected of being hypocalcaemic should be treated promptly and, if possible, before she becomes recumbent. However, the vast majority of suspected cases are recumbent when first seen by the veterinary surgeon. Before milk fever is diagnosed and drug therapy instituted, other causes of recumbency such as physical injury and infectious diseases should be suspected and ruled out as far as is possible. Therapy is based upon the parenteral administration of a warm solution of calcium salts with calcium borogluconate being the most frequently used. Optimal responses have been obtained after the administration of the equivalent of about 8 g of calcium.

Since one bottle (400ml) of a 20 per cent solution of calcium borogluconate supplies the equivalent of 6.8 g of calcium, it is advisable to use a 40 per cent solution, which will contain the equivalent of 13.5g of calcium in 400ml. It is a common practice to give half the volume intravenously and half subcutaneously. Calcium salts should be given slowly (400ml over 10-15 minutes) because of the affect of over dosage on the heart (e.g, marked tachycardia, irregular rhythm and even heart block). The value of polypharmacy preparations containing magnesium and phosphorus in addition to calcium is debatable, since demonstrable benefits have yet to be confirmed. Nevertheless, in certain instances, such solutions have been claimed to improve recovery rates.

Following treatment, many cows will eructate, defaecate and get on their feet within 15 minutes. Those in lateral recumbency will first raise itself to sternal recumbency and then stand after about two hours. Cows with milk fever should be allowed time to get to their feet on their own, because if forced, they can easily injure themselves particularly on slippery concrete floors. In prepartum cases, labour will usually recommence shortly after calcium is given. Although the affect of exogenously administered calcium will decline over approximately 6 hours, this period does allow the functions of the alimentary tract to return to normal, allowing sufficient time for adequate calcium to be absorbed from the diet.

The recovery rate in uncomplicated cases is about 75 per cent, while about 15 per cent of the total have to be culled or will succumb because of complications. About 20 per cent of apparently successfully treated cases will relapse and require a second course of therapy. If a cow does not get to its feet within three days, the prognosis is poor, because of the development of ischaemic muscle necrosis.

A small proportion of cases remain recumbent (*downer cows*) following calcium therapy and this is usually attributed to incorrect diagnosis or inadequate treatment. It has been shown that the longer the therapy is delayed after the onset of clinical signs, the lower is the recovery rate.

20.1.6 Prevention

Several regimes have been devised in order to minimise the degree of hypocalcaemia in cows at around parturition. Although most of these have enjoyed a degree of success under controlled conditions, their practical usefulness has been limited, mainly because it is difficult to forecast the exact time of parturition.

20.1.6.1 *Nutritional Management*: A high calcium diet prepartum increases the incidence of milk fever by increasing the individual's dependence on diet as a source of calcium and reduces skeletal mobilisation. Conversely, a low calcium diet (20g/day) reduces the incidence of milk fever. In hill and mid country farms in Sri Lanka, it is advisable to stop feeding mineral mixtures during last two weeks of gestation, as this practice has been shown to reduce the incidence of milk fever among dairy cows.

20.1.6.2 *Administration of Vitamin D and metabolites or analogues*: A single injection of 10 million units of Vitamin D_3 from 2 to 8 days before calving will significantly reduce the incidence of milk fever in susceptible cows. If the cow does not calve, this regime can be repeated every eight day until calving does occur. Calcification of the heart, great vessels and kidneys have been found at necropsy after this treatment regime without any apparent clinical signs of disease.

Vitamin D_3 is hydroxylated in the liver to 25-hydroxycholecalciferol and this is metabolised in the kidney to 1,25-hydroxycholecalciferol. This latter metabolite is very effective in increasing serum calcium concentrations, but it is difficult to synthesise within tissues. On the other hand, one of its analogues, 1-alpha-hydroxycholecalciferol is said to be equally effective and is easy to synthesise. Dosing is recommended at less than one week (but more than 24 hours before calving) in order to produce very good protection against milk fever. A second injection can also be given but it is recommended that this should be followed by corticosteroid treatment to ensure that the cow calves within 48 hours. This particular preparation has four main advantages over Vitamin D_3; firstly, it is highly active, secondly it is quick acting, thirdly it is not dependent upon parathormone for its action or control and finally, it raises both calcium and phosphorus concentrations in serum quickly. Its disadvantages are twofold; firstly the date of parturition may be uncertain and secondly it depresses serum magnesium concentrations. Despite these limitations, this product has been used very effectively in many countries.

20.1.6.3 *Calcium Injections*: In farms with a previous history of a high incidence of milk fever, every cow may be given 400ml of calcium borogluconate sub-cutaneously after calving.

20.2 Hypomagnesaemic tetany

Hypomagnesaemia can occur in different age categories. Firstly, the most common and important form of hypomagnesaemia affects recently calved dairy cows on lush fertilised grass in the spring (*grass staggers*). Secondly, it could affect fast growing calves which are fed wholly on milk.

20.2.1 Grass staggers (Lactation tetany)

This condition occurs in mature, lactating dairy cows usually within a few weeks of being moved on to lush grass after the first rain. The onset of clinical signs is associated with a sudden fall in the plasma magnesium and calcium concentrations and effected individuals may be found dead or in lateral recumbency.

20.2.1.1 *Aetiology*: The plasma concentration of ionised magnesium in healthy cattle ranges from 0.7-1.0 mmol/l. Although hypomagnesaemia is relatively common in lactating cows, tetany occurs only when there is also a sudden fall in the plasma calcium concentration to below 2.0 mmol/l (normal range 2.0-3.0 mml/l). Magnesium (Mg^{2+}) is an activator of enzyme systems, several of which act at the neuromuscular end plates. A reduction in the concentration of Mg^{2+} ions in the extracellular fluid increases the neuromuscular irritability, firstly, by increasing the amount of acetyl

choline released and secondly by decreasing its subsequent degradation.

Within the body, about 40 per cent of the magnesium is in soft tissues while the remainder is found in the skeleton where, in adults, it is metabolically inert. Of the total body magnesium, only the 1 per cent in the extracellular fluid is available to satisfy immediate physiological needs. An effective homoeostatic mechanism for the control of magnesium has not yet been identified. It appears that adult cattle are totally dependent upon dietary sources to satisfy the day to day requirements and to meet any sudden increase in demand.

Factors which can result in a low herbage magnesium concentration include low soil magnesium, high soil potassium, high applications of artificial nitrogenous (ammonium) and/or potash fertilisers, the composition of the herbage and the age of the sward. Factors that can reduce the intake of magnesium include absolute or relative starvation, low dry matter intake, unpalatable herbage and the sudden onset of cold wet windy weather (acute cold stress reduces both magnesium and calcium plasma concentrations). Herbage magnesium has an availability of about 30 percent, but on some diets, this can fall to as low as 5 per cent. The availability of herbage magnesium varies according to the composition of the sward, the amount of soluble carbohydrate in the diet and the amount of protein in the diet.

The major factor responsible for the sudden increase in demand for magnesium is increased milk production. The magnesium content of milk is approximately 0.5g per gallon or approximately 0.12g per litre. Approximately 20 per cent of the total exchangeable magnesium is daily excreted into the digestive tract and, consequently, anything that significantly affects resorption by decreasing the rate of passage of intestinal contents (e.g. pathological or physiological diarrhoea), will decrease the amount of magnesium resorbed. In addition, the renal threshold for magnesium is relatively low (0.7 mmol/l)

20.2.1.2 *Epidemiology*: This is a relatively common disease although the incidence varies greatly from year to year, from area to area and is closely associated with managemental and climatic factors. Clinical signs usually develop in high yielding dairy cows within the first two months of lactation (i.e. around peak milk production, within a few weeks of grazing on young, lush, fertilised grass). The incidence of the disease is said to increase with age, and it has been suggested that the incidence is highest in Ayrshires and lowest in the Jersey breed.

20.2.1.3 *Clinical Signs*: Many apparently healthy cattle can also develop hypomagnesaemia. The onset of clinical signs is frequently brought about by excitement following turning out to pasture, oestrus and transport. Although the development of clinical signs can be divided into several progressive stages, only the per-acute stage (causing death) and the acute stage (causing lateral recumbency) are relevant in this situation. In the per-acute stage, effected animals are found dead. This is one of the few conditions that can cause a cow to literally drop dead (i.e. an actual cause of "sudden death").

In the vast majority of cases, the veterinarian is presented with a cow in lateral

recumbency with the ground torn up around the animal. When lying still, there are obvious signs of opisthotonus and if stimulated (e.g. by handling), they go into convulsions and make determined paddling movements. They may also roar, foam at the mouth, defaecate, breath irregularly and often nystagmus can be seen. Consequently, on close examination, there is usually evidence of traumatic damage to the head and limbs, and the temperature is elevated. In untreated cases, death occurs relatively quickly, usually within hours of the animal becoming recumbent. The morbidity rate is low but may reach 10 per cent. The mortality rate is difficult to assess accurately because a proportion of cases are found to be dead. It is probable that about 20-30 per cent of treated animals may never recover and succumb to the disease.

20.2.1.4 *Pathology*: There are no lesions pathognomonic for grass staggers. In fact, the carcase may look absolutely normal. However, in many cases, there are sub-serosal haemorrhages, e.g. on the heart and abdominal organs. In addition, there is often evidence that the animal has been recumbent for a period, during which time it has usually caused itself traumatic damage.

20.2.1.5 *Diagnosis*: This is based almost wholly on the type of animal, management system and the time of the year. Staggers can be suspected if a lactating dairy cow is found dead in the spring, soon after being put out to grass and if hyperaesthesia and/or convulsions are seen in recently calved dairy cows, particularly if they have been grazing on lush grass for a short time after a sudden onset of cold, wet spell of weather.

At necropsy, there is a complete lack of lesions typical of other possible causes of sudden death. If the magnesium concentration in the cerebro- spinal fluid appears to remain stable for up to 12 hours postmortem, this can be used to confirm hypomagnesaemia. In contrast, the plasma magnesium concentration after death is an unreliable indicator of the animal's magnesium status in life.

20.2.1.6 *Treatment*: Suspected cases of grass tetany should be approached quietly. In order to facilitate the administration of specific drug therapy, the cow may first be sedated with a suitable sedative (e.g. acepromazine maleate, xylazine). Since hypocalcaemia is thought to precipitate the onset of clinical signs, it may be beneficial to initially administer slowly a warm solution of calcium borogluconate intravenously. When administering a solution of magnesium sulphate (25%), it is advisable to give it subcutaneously, preferably at several sites, because of the risk of ventricular fibrillation. Nevertheless, it can be given intravenously but only very slowly. Treatment with solutions containing soluble salts of both calcium and magnesium are claimed to result in improved recovery rates under certain conditions.

Clinical signs will almost certainly recur unless an adequate amount of magnesium is given in the diet because of the lack of a homoeostatic mechanism for the regulation of magnesium. Since all cows at around peak lactation are likely to be hypomagnesaemic to a greater or lesser degree, control and preventive measures must be considered from a herd point of view.

20.2.1.7 *Control and Prevention*: All control measures must be based on providing an adequate daily intake of magnesium in the ration. The most common method and the most practical is to feed concentrates with added magnesium. Also magnesium oxide as calcined magnesite can be added directly to concentrates at a rate of 50g (2 oz) calcined magnesite per cow per day. Other methods of feeding supplementary magnesium include incorporation in feed blocks, but they are considered to be of limited value because some cows never eat them. In situations where there is only a piped water supply, soluble magnesium salts can be introduced at a predetermined rate into the water supply.

The pasture itself can also be treated either in the long term with magnesium limestone or calcined magnesite which can be applied to acid soils as a fertiliser. Smaller quantities of calcined magnesite can also be dusted on herbage where it can act as a supplement during the subsequent grazing period. This is most useful in intensive grazing situations.

20.2.2 Hypomanesaemia tetany in new born calves

This is a condition which occurs in 2 - 4 moths old calves reared almost extensively on milk. It is common in fast growing calves.

20.2.2.1 *Aetiology*: The cause of the clinical signs is an absolute deficiency of magnesium due to the relatively low magnesium content of milk. The fall in the plasma magnesium concentration is progressive because in the calf, skeletal magnesium is a depletable reserve. The drop in plasma magnesium is exacerbated in fast growing animals and, as the calf gets older, not only does its requirement for magnesium increase but the efficiency of absorption from the alimentary tract also decreases. The feeding poor quality roughage also decreases the efficiency of absorption of magnesium.

20.2.2.2 *Clinical Signs*: Clinical signs are similar to those in dairy cows with grass staggers and frequently the onset of clinical signs are initiated by excitement. e.g. during collecting calves for routine examination. Calves are commonly found dead usually with some evidence of their having had convulsions.

20.2.2.3 *Treatment*: Treatment with magnesium sulphate subcutaneous has been proved to be successful.

20.2.2.4 *Prevention*: The best method is to offer creep feed to calves as soon as possible. Early weaning and the use of supplement of milk substitutes is also important in preventing hypomanesaemia in calves.

20.3 Acetonaemia (ketosis)

This is a disease of ruminants which is the result of a failure to metabolise carbohydrates and volatile fatty acids and is characterised by high blood ketone and low blood glucose levels. This condition is usually seen in high producing dairy cattle in the first two months of lactation.

All high yielding dairy cows are in negative energy balance in early lactation and are, therefore, biochemically if not clinically ketotic. This 'biochemical' ketosis can readily turn to a 'clinical' ketosis by relatively minor dietary or metabolic problems, when the demand for energy exceeds that available from normal glucose, glycogen sources and hepatic gluconeogenesis. This results in a rise in ketone body formation which exceeds the level that is normally tolerated.

Any reduction in propionic acid production leading to inadequate oxaloacetate levels results in ketone body production, and any excess in dietary protein intake will exacerbate the problem. Many theories have been put forward in an attempt to find causal factors including adrenal, thyroid and hepatic insufficiency. The composition of rations (e.g. silage is potentially more ketogenic than hay), ruminal flora and other abnormal ruminal conditions may play a part in the development of clinical ketosis.

20.3.1 Epidemiology

Ketosis is seen in dairy cattle especially during the winter in temperate countries. The incidence has been estimated in UK to be between 1 and 5% in all dairy cows but the economic loss is probably underestimated because it is non-fatal in nature. There are production losses both during the course of the disease and from subsequent effects on the milk yield during lactation. The economic affect of subclinical ketosis may be almost as severe as that of the clinical disease. However, this condition has not been confirmed in Sri Lanka, although administration of glucose has been a common practice. The absence of this condition in Sri Lanka most likely may be due to modest production levels attained by dairy cows. In addition to the primary disease, ketosis may occur as a complication in some deficiency diseases, especially in cobalt deficiency states and in other conditions resulting in inappetence.

20.3.2 Clinical findings

Clinically ketosis could fall either into a wasting form or a nervous form.

20.3.2.1 *'Wasting' form*: In this form, there is a gradual reduction in appetite with selective anorexia (e.g. refusal to consume concentrates, while the animal continues to eat roughages) and a concomitant reduction in milk yield over several days. Weight loss may be quite dramatic over this period. Faeces will be firm and dry, often "horse-like", ruminal movements will be reduced in frequency and intensity, and the cow appears to be depressed. Temperature, cardiovascular and respiratory parameters are all within the normal range. There is a smell of ketones in the breath and in the urine. Sometimes the appetite becomes depraved and occasionally transient nervous signs (usually staggering) are seen. The animal in the wasting form will recover spontaneously without treatment but as this takes about 4 weeks. In order to minimise production losses, which are high, animals are usually treated.

20.3.2.3 *Nervous form*: The nervous signs are bizarre and of sudden onset, often occurring between periods of apparent normality. The cow appears delirious, walks in circles, crosses her legs, leans on objects and has licking and chewing manias either involving herself or adjacent inanimate objects. They can often be seen chewing

concrete steps or tubular cubicle divisions. They also have a depraved appetite, make chewing movements, salivate profusely, become hyperaesthetic and very noisy when stimulated. Self-inflicted trauma is common in the nervous form of the disease.

20.3.3 Diagnosis

Clinical signs and the history, together with the exclusion of other conditions where ketosis might be a secondary manifestation (e.g. left displacement of the abomasum) are usually adequate. Biochemistry will show hypoglycaemia, ketonaemia and ketonuria, and the presence of ketones in milk.

20.3.4 Treatment

Administration of intravenous glucose produces a rapid response in all cases and is essential in 'nervous' cases, but unless treatment is repeated most cases will relapse. Oral glucogenic agents may be given (e.g. propylene glucol or glycerine) especially to animals in the 'wasting' form but 'nervous' cases should be maintained on intravenous glucose until the nervous signs disappear.

Corticosteroids are of great value in maintaining the immediate response following intravenous glucose by stimulating gluconeogenesis. Betamethasone and dexamethasone are the steroids of choice. Anabolic steroids (e.g. trenbolone acetate) may also be used.

20.3.5 Prevention

Prevention is achieved by proper management of the prepartum cow. Cows should be fed adequately during the prepartum period and the body condition score should be maintained at around 3. The roughage diet should be of good quality and the protein content of the concentrate ration should not exceed 18%. If possible cows should be made to exercise.

Selected references

Kronferld, D.S. (1971) Pathogenesis of Hypocalcaemia. *Advances in Veterinary Science and Comparative Medicine.* **15**, 133 - 137.

Roggers, P.A.M. (1979) Hypomagnesaemia and its clinical syndrome in cattle: A review . *Irish Veterinary Journal.* **33**, 115 - 124.

Sansom, B.F. Manston, R. and Vagg, M.J. (1983) Magnesium and milk fever. *Veterinary Record.* **112**, 447 - 449.

Stober, M. and Dicksen, G. (1982) The recumbent cow: differential diagnosis and differential therapy. *Veterinary Annual.* **22**, 81 - 99.

Chapter 21

Differential Diagnosis of Common Bovine Diseases

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Introduction

In this chapter, an attempt has been made to provide a reference guide to help veterinary practitioners to make a quick disease diagnosis by carefully analysing the common clinical signs with those of other diseases which may present similar clinical signs. The diseases listed under each clinical manifestation are those already reported/observed in this country or those that are believed to or likely to be present, though not confirmed as yet. The text has been deliberately made concise, giving only the key points under each disease, so as to allow the practitioner the opportunity for quick reference. The descriptions of bacterial and viral diseases in bovines in Chapter 22 are augmented by this chapter. Nonetheless, should the reader require further information on diseases mentioned here or more descriptions of the conditions listed, he/she is directed to text books in large animal medicine given in the list of references at the end of the chapter.

21.1 Clinical manifestations of ABORTION

21.1.1 *Brucellosis*: A common disease in Sri Lanka. Abortion usually in the last trimester. Retained placenta and infertility may occur as a sequelae to chronic metritis. Hygromas may be present in infected males and females.

21.1.2 *Trichochomoniasis:* Abortions at three to four months. Endometritis with yellowish-white mucopurulent discharge is common, motile trichomonads on fresh microscopic examination, mucopurulent discharge from prepuce with small red nodules on penis.

21.1.3 *Vibriosis:* Abortions usually at three to six months; endometritis. Organism recovered from vaginal mucus.

21.1.4 *Leptospirosis:* High temperature, no obvious change of demeanour, jaundice, blood stained milk, anaemia. Abortion usually between four and nine months.

21.1.5 *Listeriosis*: Rare cause of abortion, but if present abortion at six to nine months, sometimes full-term dead foetus. Recover *Listeria monocytogenes* from stomach and brain and pleural cavity of foetus.

21.1.6 *Non-specific genital infections*: *Actinomyces pyogenes*, streptococal infection, *E.coli* infections, have also been associated with abortion in cattle.

21.1.7 *Trauma/mechanical injury*: History of falling down a bank or similar incident; transport over a very long distance, particularly on 'bumpy roads'.

21.1.8 *Pyrexia*: Most clinical diseases with severe pyrexia is often associated with abortion e.g. babesiosis, Ephemeral fever.

21.1.9 *Poisoning*: Acute poisoning e.g. heavy metals, pesticides, weedicides, induce abortion.

21.1.10 *Foot-and-mouth disease (FMD)*: Clinical evidence of FMD include oral and foot lesions characterised by vesicles or raptured vesicles leaving a raw erosive areas; smacking of lips, drooling sticky foamy saliva, lameness, sudden drop in milk production, rapid loss of body condition; rapid spread of disease in the herd.

21.2 Clinical manifestations of **ANAEMIA.**

21.2.1 *Nematode infections*: Heavy worm infection especially, abomasal worms (haemonchosis), hook worms (Bunostomum). Examine faecal smear for parasitic eggs.

21.2.2 *Coccidiosis*: Important in calves. Blood stained diarrhoeic faeces between 20 and 30 days of age, tenesmus, general weakness and debility. Examine faecal smear for oocysts.

21.2.3 *Babesiosis*: Common in the up and mid country areas. Raised temperature, anorexic, drop in milk, panting movements with breathing, ears droop, red/light coffee colour urine, particularly in *Babesia bigemina* infection, where the organism can be demonstrated on peripheral blood smears. *Babesia bovis* infection is more rapid and more severe. The latter may be associated with nervous signs, such as inco-ordination. Jaundice may be present in the latter part of the disease.

21.2.4 *Haemorrhage*: History of severe haemorrhage from a wound or surgical incision, bleeding from internal organs such as the gut (abomasal ulceration, parasitic infection), urinary bladder (bracken fern poisoning, tumours), haemorrhage at calving or dystocia. Clinical signs include pallor, breathlessness, weakness, shock and collapse.

21.2.5 *Haemolysis*: Diseases characterised by haemolysis e.g. leptospirosis, *Clostridium haemolyticum* infection.

21.2.6 *Acute paramphistomiasis*: Immature paramphistomes in the small intestines. Signs include diarrhoea, hypoproteinaemia, dehydration and weight loss. Examine diarrhoeic contents for immature flukes.

21.2.7 *Tick infestation*: Very heavy tick infestation of blood sucking ticks such as Boophilus spp. and haemophysalis.

21.3 Clinical manifestations of **COLIC or ABDOMINAL PAIN**

21.3.1 *Indigestion and ruminal atony*: In animals on grain/concentrate feed or after consumption of large quantities of boiled rice from garbage cans or dumps. Sudden onset, anorexia, No cudding, marked drop in milk yield, no ruminal movements. Death often follows. Necropsy; signs of bloat, undigested carbohydrate material e.g. grains of rice in ruminal contents. Histology; Chemical ruminitis.

21.3.2 *Peritonitis:* Stands still, anorexia, increased pulse and temperature, expression of pain on palpation.

21.3.3 *Traumatic reticulitis*: Pinching on the cows back may cause crouching and grunting, abdominal muscles rigid, anorexia, pain when upward pressure is applied behind the xiphoid area.

21.3.4 *Traumatic pericarditis*: Signs of heart failure (e.g. brisket oedema, pulsation and engorgement of jugular veins), muffled heart sounds. Abducted elbows, reluctant to move, especially down a slope. Prefers standing. Faeces and urine is passed in large volumes in between long intervals.

21.3.5 *Ruminal impaction:* Paddling feet, anorexia, decreased milk yield, grunting and groaning, increased pulse, firm mass in the rumen. Reduced ruminal movements. Mild cases recover spontaneously. Severe cases need attention.

21.3.6 *Displacement of abomasum*: Ping sounds (typically high pitched with tinkling and splashing sounds which are often peristaltic) over the dorsal two thirds of the left rib cage (between 9th and 12th ribs) on special percussion. Intermittent colic. Selective anorexia (eats roughage but refuses concentrates), marked drop in milk, rumen movements reduced, faecal output reduced, varying consistency; sometimes intermittent profuse diarrhoea. Clinical signs in right-sided displacement is more severe than that of the left side.

21.3.7 *Abomasal ulceration*: Signs of indigestion, sometimes colic, Depraved appetite - eating dung, bedding. Dehydration and emaciation, malena.

21.3.8 *Bloat*: Marked abdominal distension particularly on the left side, gasping for breath, congested/cyanotic ocular and oral mucus membranes.

21.3.9 *Intestinal obstruction*: Rare. May arise from intussusception or strangulation of small intestines. Intense colic, kicking at belly, rolling on ground, grunting. Rectal examination may reveal fluid filled intestinal loops.

21.3.10 *Uterine involvements*: Torsion of uterus, rapture of uterus etc. History of pregnancy; per vaginal or per rectal palpation helps confirmation.

21.4 Clinical manifestation of **EYE DISCHARGE**

21.4.1 *Pink eye*: Usually a herd disease, common during dry periods, inflammation of conjunctiva; photophobia; cornea may become opaque with new blood vessels developed over – pink eye, purulent discharge, temporary blindness. Isolate of *Moraxella bovis* from eye swabs.

21.4.2 *Occular cancer*: Rare in Sri Lanka. Unpigmented skin around eye; tumour mass may vary from a nodule to that of a structure covering the entire eye.

21.4.3 *Photosensitisation*: Grazing on sensitisation plants such as lantana. Examine for other signs of photosensitization e.g. facial eczema, alopecia of areas on the head, back etc.

21.4.4 *Foreign body*: Grass seeds, twigs or other foreign matter. Usually on one eye.

21.4.5 *Vitamin A deficiency:* An uncommon possibility. May occur in the absence of green fodder during drought periods. Night blindness, sensitive to bright light. Opaque cornea or ulceration of cornea, rough coat.

21.4.6 *Eye worm*: Requires close examination of the eye; Thelazia spp. (2-3 cm, thin, thread-like worm) present in conjunctival sac.

21.5 In the case of **SUDDEN DEATH** look for the following.

21.5.1 *Haemorrhagic septicaemia*: High temperature, inappetance, submandibular oedema, salivation, watery nasal secretion, respiratory distress. In non-endemic areas, all ages groups affected; in endemic areas usually 6m to 2 year group. Buffaloes more susceptible..

21.5.2 *Poisoning*: Particularly important are weedicides and pesticides consumed in large quantities. Signs differ with each type of poison. Confirmation is by chemical analysis of ruminal contents.

21.5.3 *Anthrax*: Clinical signs include, high temperature, sudden death, inappetance, abortion, bloody discharges from mouth, nostrils and anus; blood stained urine. Anthrax has not been reported in Sri Lanka since 1969. However spores are known to remain viable in the soil for decades.

21.5.4 *Acute salmonellosis:* In young calves, septicaemia, scouring and sudden death. In older calves scouring with blood.

21.5.5 *Blackquarter*: Lameness in one or more legs, hot painful, local swelling, crepitation on palpation, raised temperature.

21.5.6 *Bloat*: Frothy bloat following consumption of bloat inducing-plant material eg. young legumes or bloat may be secondary to ruminal atony e.g. consumption of toxic plants, e.g.

Magnihot glaziovii (Cearra rubber), hypocalcaemia, tetanus. Marked abdominal distension, respiratory distress, injected/cyanotic ocular or conjunctival mucus membranes.

21.5.7 *Snake bite*: Swelling of the area stung by snake. Staggering gait. History of snakes in the area or incidents of previous snake bites.

21.5.8 *Lightning strike*: Not common. History of lightening and thunder. Often animals in open field with a chain are affected. Burn marks visible on the body hair.

21.5.9 *Aspiration of ruminal contents or medications*: History of aspiration e.g. during drenching or passing of stomach tube. Respiratory distress.

21.5.10 *Electrocution:* Evidence for contact with a "live" cable/wire. Burn marks on the body where the wire had come into contact.

21.6 Clinical manifestation of **WASTING or PROGRESSIVE LOSS OF WEIGHT**

21.6.1 *Malnutrition*: The most common cause of wasting, history of deficiency of food (drought, floods) or unsuitable feed (unpalatable, lacking nutrition e.g. straw, etc.). Particularly manifested in growing animals and those in pregnancy or lactation.

21.6.2 *Tuberculosis*: Asymptomatic but positive to the tuberculin test. Advance cases – wasting, scouring, coughing, swelling of superficial lymph nodes. Rough coat. Rare in Sri Lanka.

21.6.3 *Johne's disease:* Rare, progressive wasting, submandibular oedema, emaciation, drop in milk, intermittent diarrhoea. Occurs mainly in adults.

21.6.4 *Neoplastic growth*: Tumour on body, wasting.

21.6.5 *Foreign body:* Traumatic reticulitis or pericarditis. (see section 21.3.4)

21.6.6 *Parasitic infestation*: Chronic abomasal and intestinal parasitic infections.

21.6.7 *Abomasal ulceration*: Intermittent diarrhoea, wasting malena

21.6.8 *Actinobacillosis*: Hardening of tongue, ulcer on the surface or enlarged superficial lymph nodes with purulent discharge.

21.6.9 *Actinomycosis*: Asymmetrical swelling of the face closely associated the bones of the maxilla or the mandible. Affected bones are rarefied and spongy with cavities filled with pus, enlargement of the facial bones. In advance cases, loosening of teeth and sinuses may be present on the skin over the swelling through which thick, sticky yellowish pus containing pale yellow granules appear ¥ sulphur granules.

21.6.10 *Septic condition*: Abscess or other septic wounds on the body. Septic metritis.

21.6.11 *External parasitic infestations*: Heavy tick and lice infestations can cause progressive loss of weight.

21.6.12 *Coccidiosis*: Particularly in calves, blood stained faeces, wasting, unthrifty, anaemic. Oocysts in faecal smear.

21.7 Clinical manifestations of **SWELLING OR LUMPS** on the body.

21.7.1 *Actinomycosis*: Swelling on the face. Also refer section 21.6.9.

21.7.2 *Neoplastic growths*: Malignant or begin tumour growths on any part of the body.

21.7.3 *Foreign body*: Deeply embedded foreign bodies give rise to abscess formation and localised swelling.

21.7.4 *Warts*: Masses of cauliflower-like growths, usually occur on the head, neck, shoulders or back of the animal. Bleeding could occur from tissues between warts. These areas may also become maggot infested.

21.7.5 *Insect stings*: Insects stings are often associated with swelling. Obvious when on face.

21.7.6 *Haematoma*: Blood clot in the muscles and subcutaneous tissue often following tissue injury.

21.7.7 *Snake bite:* Localised swelling at the site of bite, nervous signs, sometimes haemoglobinuria.

21.7.8 *Urticaria:* Weal-like swellings. Focal or diffused.

21.8 Clinical manifestations of **JAUNDICE.**

21.8.1 *Babesiosis*: Jaundice is present in the latter stages of babesiosis. Also refer section 21.2.3.

21.8.2 *Anaplasmosis*: Temperature, anaemia, jaundice in the latter stages of the infection. Parasites on blood smears. Necropsy - spleen enlarged, heart may show haemorrhages.

21.8.3 *Leptospirosis*: Red urine, mainly affects calves or young animals, sometimes adults. High temperature, anorexic, jaundice, pale mucous membrane. Abortion.

21.8.4 *Photosensitisation*: Caused by grazing on plants such as lantana. Facial eczema, ulcerated areas on the skin. Discharge from eyes and nose. Jaundice arises from liver damage.

21.8.5 *Post-parturient haemoglobinuria*: Usually in animals between 4 to 8 years. Occurs one to three weeks after calving. Red urine, increased heart and respiratory rates, anaemic.

21.8.6 *Liver damage*: Liver pathology results from poisoning and/or multi-focal or diffused abscesses, liver infections. Jaundice may be associated with emaciation. Scouring may be present.

21.9 Clinical manifestations of **COUGH**

21.9.1 *Tuberculosis*: Seldom reported in Sri Lanka. Positive reactors to the tuberculin test without other signs. Advance cases - wasting, scouring, coughing, swelling of superficial lymph nodes, rough coat.

21.9.2 *Aspiration*: Associated with faulty drenching or choking while eating dry food.

21.9.3 *Pneumonia*: Coughing is a common clinical sign which is associated with pneumonia caused by various aetiological agents. Pneumonia is often associated with nasal discharge, raised temperature, anorexia, profound depression, failure to grow and sometimes death. Necropsy will reveal consolidation of lungs and other complications, such as abscesses, emphysema and collapse.

21.9.4 *Lung abscesses*: Parasitic invasion, bacterial infection, foreign body penetration may result in chronic cough. Epistaxis and haemoptysis, mild fever may also be present.

21.9.5 *Obstruction in the throat*: Large food particles, e.g. Jak skin stuck in the pharynx or oesophagus.

21.9.6 *Calf diptheria*: Coughing and gasping in calves. Raised temperature, affects individuals, necrotic lesions in tongue, mouth and larynx..

21.9.7 *Neoplasms*: Neoplasms in the throat, larynx or lungs will result in cough.

21.9.8 *Laryngitis, Phayngitis or Tracheitis*: Inflammation of larynx, pharynx or trachea by specific infections or non-specific causes such as irritant material, dust or smoke will cause cough.

21.10 Clinical manifestations of **HAEMATURIA**

21.10.1 *Bracken fern poisoning*: Occurs in cattle in the mid and up country regions. Red urine, anaemia, pyrexia, jaundice, loss of weight (also refer section 21.2.4).

21.10.2 *Uterine conditions*: Recent calving, may associate retained placenta.

21.10.3 *Calculi*: May occur in the renal pelvis, bladder or other parts of the urinary tract.

21.10.4 *Pyelonephritis*: Blood and pus in urine. Enlarged kidneys, pain on per rectal palpation. Loss of condition, drop in milk, ureters and bladder thickened and inflamed. Confirmation by isolation of *Corynebacterium renale* in urine.

21.11 Clinical manifestation of **HAEMAGLOBINURIA**

21.11.1 *Babesiosis*: Deep red or brown colour, no red cells or cell debris on sedimentation (also refer section 21.2.3).

21.11.2 *Post-Parturient haemoglobinuria*: Usually occurs in cows (4-8 years) one to three weeks after calving.

21.11.3 *Bovine bacillary haemoglobinuria*: Sudden onset. jaundice, raised temperature, dull, salivation, dark tarry faeces, abdominal pain, grunting, pregnant animals may abort. Necropsy – acute inflammation of abomasum, blood stained intestinal contents; recover *Clostridium oedematiens*.

21.11.4 *Leptospirosis*: Mainly affects calves and young animals. Calves raised temperature, anorexia, pale mucus membranes, short clinical course. Chronic cases show jaundice, wasting and poor growth rates.

21.11.5 *Phosphorus deficiency*: In acute phosphorus deficiency. Wasting, lameness and depraved appetite are other clinical signs.

21.11.6 *Cold water haemolytic anaemia*: A rare condition, but may occur under special circumstances. The classical history is where calves have been starved for water and allowed access to a large volume of very cold water. Sudden onset. Acute haemolytic anaemia may follow death within a few hours.

21.12 Clinical conditions associated with **TEAT AND UDDER SKIN LESIONS**

21.12.1 *Udder impetigo*: small 2-4 mm pustules on the teat and on the udder skin. Spread from cow to cow by milker's hands. May disappear spontaneously or recur and persist in a herd; *Staphylococcus aureus* on culture.

21.12.2 *Teat fibropapilloma:* Flat small elevated (0.3 cm) plaques or horny wart like tags about 1 cm long. Spread from cow to cow. Tend to disappear in two to five months.

21.12.3 *Udder injuries*: Injuries may result from barb wires, treading on teats and horn injuries.

21.12.4 *Photosensitisation*: Feeding on plants containing photodynamic agents e.g. lantana or fungal infected feed. Photosensitization reactions of the skin are manifested on the teat and udder skin. Similar lesions may also be seen around the eyes and nose. The lesions on the skin

causes reddening, scabbing and shedding of the skin over the teats. Animals with these lesions are sensitive to milking.

21.12.5 *Cracked teats*: Transverse and longitudinal cracks on the teats. Painful at milking. Often associated with faulty milking.

21.13 Differential diagnosis of common **CALF DISEASES**

21.13.1 *Worm infestation*: Stunted growth, anorexic, anaemic. Listless and lethargic. May have diarrhoea. Rough coat. Parasite eggs in faecal smear.

21.13.2 *Calf scours*: White scours usually occurs from birth to 14 days but may extend up to 30 days. Watery, yellowish, adheres around tail, perineum and upper part of rear legs. Dehydration. Naval infections and arthritis are frequent complications. Death may occur if unattended.

21.13.3 *Calf pneumonia*: Usual occurrence between two weeks and four months. Coughing, depression raised temperature, rapid breathing, nasal discharge, sunken eyes, anorexia. Necropsy indicates consolidation of lungs.

21.13.4 *Enterotoxaemia*: Age occurrence may vary from about three months to two years. Sudden death. Bloating, nervous signs, head down. Necropsy - inflammation of intestines.

21.13.5 *Tetanus*: Deep wound or after surgical intervention e.g. castration, dehorning. Stiffness of limbs, protrusion of third eye lid, bloating, erected ears. tetanic spasms on excitement. Rare condition in cattle.

21.13.6 *Ringworm*: Circumscribed dry crusty alopecic patches, particularly on the face and neck regions. A herd problem, especially in animals on poor nutrition.

21.13.7 *Leptospirosis*: Jaundice, red urine, high temperature, anorexic, pale mucous membranes. Affected calves become prostrate and die in 24 to 48 hours. Chronic cases show high mortality, jaundice, wasting, and sometimes nervous signs such as convulsions.

21.13.8 *Warts*: Affects calves of all ages. Small raised cauliflower-like lumps especially around the eye, neck and back regions. May heal on its own or spread to other parts of the body and become complicated with secondary bacterial or maggot infection.

21.13.9 *Calf Diptheria*: Ulceration of the mouth and throat, often passes unobserved in clinically sick animals. Unpleasant breath, raised temperature, coughing. High death rates in affected calves.

21.13.10 *Black quarter*: Sudden death in calves up to two years. Lameness in one or more limbs. Affected limb swollen, hot, crepitation on palpation. Raised temperature. Necropsy

shows dark red colouration of affected muscles with blood stained fluid oozing on cut surface.

Further reading

Andrew, A.H., Blowey, R.W., Boyd, H and Eddy, R.G. (1992) (Eds.) *Bovine Medicine B Diseases and Husbandry of cattle.* Blackwell Scientific Publication.

Boden, E. 1991(Ed.) *Bovine Practice*. Bailliere Tindall, London, UK.

Hungerford, T.G. (1990) *Hungerford=s Diseases of Livestock.*. (9th edition) McGraw-Hill Book Company, Sydney, Australia

Radostits, O.M., Blood, D.C. and Gay, C.C (1994) (Eds.) *Veterinary Medicine.* (8th edition) Baillere Tindall. London, UK

Sewell M.M.H. and Brocklesby, D.W. (1990) (Eds.) *Animal Diseases in the Tropics.* (4th edition) Bailliere Tindall, London, UK.

CHAPTER 22

Necropsy Procedure in Bovines

N. U. Horadagoda

Introduction

The methodical examination of organs and tissues of the dead animal is called either necropsy (Greek; *necros*, dead body: *opsis*, sight) or autopsy (Greek; *autopsis*, seen by oneself). The term necropsy is preferred in veterinary pathology; autopsy is used in medical pathology. The objectives of a necropsy in veterinary practice may be (a) to determine the cause of death, (b) to clarify the clinical diagnosis of a disease or (c) to define more clearly the type and extent of lesions causing clinical disease. In some situation, the objective of the necropsy may be limited and this may be achieved by a partial necropsy examination. Nonetheless, it has to be emphasised that a well performed necropsy can be highly informative while a necropsy that is not well prepared and haphazardly carried out can be a waste of time that may even lead to an erroneous diagnosis. This chapter on necropsy procedures in bovines is written for the relatively inexperienced veterinarian and is focussed on a method that could be used well within a farm environment.

22.1 Instruments

One of the important aspects of getting ready for a necropsy is to make sure that one has the minimum of the instruments required. Table 1 shows a check list of items needed.

Table 1. Minimum travelling necropsy kit

Knife	Rubber gloves
Scissors	Note book pen/pencil
Tissue and bone forceps	Coverall
Saw	Rubber boots
Sampling bottles and swabs	Disinfectant

22.2 General comments

It is always desirable to advise the farmer to ensure that the carcass is presented in as fresh a state as possible in order to make the best interpretation from a necropsy. The veterinarian should ensure that there is adequate light and running water at hand, as viscera and instruments frequently need to be washed during the procedure. Some thought should be given to the suitability of the site with regard to disinfection and for the safety of all concerned. Further, it is advisable to plan the disposal of the carcass prior to commencement of the necropsy.

22.3 History

Before beginning a necropsy, it is imperative that the veterinarian should read the

clinical history or the anamnesis carefully, and if necessary, obtain further information from the owner. This will enable him to direct special attention to the organs which have given rise to clinical signs thereby shorten the necropsy time.

22.4 External examination

Prior to making any incisions on the carcass, it is important that a thorough systematic external examination (including orifices) be carried out. Observe any evidence of nasal exudates, muzzle or oral ulcers, eye lesions, lesions of the ears or skin, foot lesions, swollen joints, hair loss, umbilical hernias, omphalitis, evidence of diarrhoea and other changes. Note the general body condition, structural abnormalities and examine the oral and conjunctival mucous membranes for any discolourations. Note the sex and age of the animal. If the carcass is not fresh, *rigor mortis* may be evident and a rough idea of the time of death can be estimated. The earliest change, especially a few hours after death is likely to be discolouration of intestines. Much later, when rigor has passed off, general decomposition will be quite advanced, post mortem bloat is likely to be present and many organs will show gross autolysis. Between these stages, a gradual decomposition will take place in which the abdominal contents will tend to decompose before the thoracic organs. Cold climatic conditions will delay the onset of post-mortem changes, while warm weather will expedite the commencement of these changes and hence the recent weather conditions should be considered when assessing the onset of autolytic and putrefactive changes.

22.5 Primary incision

The animal is positioned with the left side down, i.e. the head should be on one's right as one approaches it from the ventral surface. Although there are other ways to position the animal, this approach where the rumen is down, works well and is the basis of the description given below.

Begin the necropsy by reflecting both right front and right rear legs. Once the legs are reflected, extend a mid-line skin incision from mandible to pubis and reflect the skin dorsally to the level of the vertebral processes of the spinal column. Also reflect the skin ventrally beyond the midline to expose the subcutaneous tissue around the umbilicus. In females, remove the udder. In the case of males, the penis and prepuce are drawn backwards to avoid damage when the abdominal wall is incised. This is done by first incising the parietal tunica vaginalis and then extending the incision as far as the external inguinal ring followed by dissection to free the penis and prepuce as far back as the ischial arch.

At this point in the dissection, examine the subcutaneous tissue for any discolouration and the amount of fat. The inguinal, subscapular and other superficial lymph nodes need to be examined for enlargement, haemorrhage, oedema or discolouration. Also note the changes in the genitalia.

22.6 Opening of the body cavities

The abdominal cavity is opened by cutting the abdominal musculature (carefully if

bloated) along the caudal margins of the ribs. Continue this incision along the ventral midline and reflect the abdominal musculature caudally and dorsally towards the pelvis.

To open the thoracic cavity, insert the necropsy knife into the thoracic cavity through the diaphragm and pull upwards in the region of the costosternal junctions. Locate the soft cartilage of the costochondral junction and advance the incision cranially along the costochondral junction. Reach in and incise the diaphragm before reflecting the ribs. The rib could be removed by pushing each or a group (two or three) ribs dorsally.

After the thoracic and abdominal cavities are opened, examine the organs *in situ* for lesions on visceral organs and for evidence of any displacements such as herniation, torsion or abomasal displacement. Note the presence of effusion in the body cavities and for evidence of inflammatory changes of the pleura and peritoneum. Heart blood can be collected at this stage. Unilateral pulmonary hypostatic congestion may be noted.

22.7 Removal and examination of the thoracic viscera

Examine the oral cavity (*rigor mortis* may restrict opening) and note any changes on lips, teeth, tongue and the colour of the oral mucosa. Remove the tongue by cutting along the inner aspect of the mandibles, around the pharynx and through the hyoid bones. Insert an index finger into the oral cavity through one of these incisions and if possible, pull the tongue ventrally to the exterior. There may still be anterior attachments of the tongue in the region of the mandibular symphysis. Use the tip of the necropsy knife to cut these anterior attachments while pulling the tongue ventrally to reflect it.

Cut the articulation of the laryngeal cartilages with the thyrohyoid (or hyoid) bone and reflect the larynx and anterior oesophagus towards the thoracic cavity by pulling on the tongue with the left hand. In very young animals, the hyoid bones and the thyroid cartilages are poorly calcified and can be easily cut with a knife. In adult cattle and buffaloes, the cartilages and hyoid bones are more calcified, and it is necessary to slide the knife into the "V" formed by the cartilages of the hyoid bones and the projections of the thyroid cartilages. Approach this junction from the caudal side. The knife should be slipped into the soft cartilaginous junctions and cut. Locate the tonsils before reflecting the trachea and oesophagus for these may be needed for microbiologic examination. In cattle and buffaloes, the tonsils are found in crypts in the lateral posterior pharynx.

Continue reflecting the trachea and oesophagus to the thoracic inlet; note the size of the thymus. Loosen the lungs and pericardium from their attachments by making incisions (a) along the ventral margin of the spinal column, (b) along the dorsal margin of the sternum (be careful not to puncture the pericardial sac) and (c) along the cranial surface of the diaphragm. Place a ligatures on the thoracic side of the oesophagus and posterior vena cava as they pass through the diaphragm and incise cranial to ligature. Lift the lungs and heart out of the thoracic cavity along with the attached trachea,

oesophagus and structures of the pharynx and oral cavity. Place the thoracic viscera on a table or a suitable surface such as a clean polythene sheet for further examination.

22.7.1 Examination of the lungs

Begin the examination of the thoracic viscera by opening the entire length of the oesophagus with either the tip of a knife or scissors. Then return to the larynx and open the trachea along the dorsal cartilaginous junctions to the level of the tracheal bifurcation and continue into the main bronchus of several lobes. Examine the respiratory lymph nodes, particularly the tracheobronchial and mediastinal nodes. Following the examination of the airways, slice through the remaining lung in any area that appear to contain lesions; look for exudates, parasites or other evidence of disease. After the lungs have being examined, open the pericardial sac and note the nature and volume of the contents.

22.7.2 Examination of the heart

There are several methods of opening and examining the heart, one of which is described here. Position the heart with the left ventricle on the right and the right ventricle on the left with the left longitudinal coronary groove running vertically at an angle from your left to your right. Incise the walls of the right and left ventricles parallel to the left coronary artery. The first incision will be into the left ventricle extending through the left artrioventricular valve, and the other incision will enter the right ventricle and extend through the pulmonary valves. Examine the valves and then make two cuts perpendicular to the first incisions. On the right side of the heart, this incision will carry through the right artrioventricular valve (tricuspid). On the left side of the heart, the knife will slide under the artrioventricular valves, into and opening the aortic valve. Examine the ventricular septum for defects and the *ductus arteriosus* (ligamentum arteriosum) for patency. Examine the moderator bands and papillary muscles and finally slice through the myocardium in several areas, looking for haemorrhage, mineralisation or scarring.

22.8 Removal of abdominal viscera

The spleen is not usually visible in the normal animal necropsied on the left side. The tip of the spleen may often be seen extending around the liver at the ventral midline if splenomegaly is present. Remove the omentum before reflecting the intestines. Locate the duodenum and place two ligatures 2-3 inches apart in the proximal section. Cut between these ligatures and remove the spleen and forestomachs (including abomasum) out of the carcass while freeing the attachments to the abdominal wall and liver. Place a further ligature in the descending part of the duodenum and at the rectum. Remove the entire intestines between these ligatures. Sever the attachments of the intestines to the root of the mesentery by blunt dissections. The pancreas, liver and the portion of the duodenum in the abdominal cavity should be removed together. Organs should be removed as neatly as possible without damage to neighbouring structures and kept until the necropsy is completed.

22.9 Removal of pelvic viscera

The floor of the pelvis is removed by sawing from the anterior and posterior borders through the obdurator foramen on each side (do not saw too far laterally). In younger animals, the pelvic symphysis can be split with a necropsy knife. The penis has meanwhile been freed from the ischial arch. The kidneys and adrenals are freed together and by carefully pulling them backwards, the ureters will be freed from the dorsal abdominal wall as far backwards as the urinary bladder. The urogenital organs, adrenals and rectum are removed together.

In females, the broad ligament is incised along its insertion on the abdominal wall so that the ovaries, oviduct and uterus will accompany the kidneys and ureters. All these organs are gathered into the left hand and then are drawn backwards at the same time as the attachments to the pelvic walls are cut through by cautious use of the knife. An incision around the anus completely frees these organs.

22.10 Examination of abdominal and pelvic viscera

22.10.1 Urogenital organs

The kidneys are sectioned longitudinally from the convex surface towards the pelvis and the capsule is removed. The ureters are opened while the adrenals are divided through many sections. The urinary bladder is opened along the ventral surface (save urine; it may be needed for chemical or toxicological examination) and the incision is continued out along the urethra. In females, the vagina and vulva are opened along the ventral border and the incision continued anteriorly through the cervix and into the uterus. If there are abnormal contents in the uterus, a small segment is isolated between ligatures and retained unopened for bacteriological examination. The ovaries are incised. Each half of the udder as well as the mammary lymph nodes are sectioned. In males, the testes are incised to examine the cut surface.

22.10.2 Spleen

The spleen is incised through several longitudinal, parallel incisions made a few centimetres apart.

22.10.3 Forestomachs and abomasum

Open the abomasum and forestomachs along the greater curvature. Examine the contents and retain material if needed for special examinations, such as toxicology. Observe the mucosae for lesions such as erosions or ulcerations.

22.10.4 Intestines

It is always desirable to open the alimentary tract last in order to minimize contamination of the post-mortem site. To open the intestine, first cut the mesentery at its attachment to the intestine with the help of a shape knife or scissors. The lumen of the intestine is opened along the mesenteric attachment thus allowing a clear view

of the gut associated lymphoid tissues (Peyer's patches). The extent of the intestine to be opened will be dependent on the disease and the clinical manifestations. If the animal does present evidence of enteritis it may be necessary to open the entire length of the intestines but in most cases opening areas that look grossly abnormal and spot-checking a few areas of the jejuneum and ileum would suffice. Collect certain regions of the intestines, such as the duodenum, jejunum, ileum and mesenteric lymph nodes, for culture. Select these specimens before landmarks are destroyed or displaced. Locate the duodenum by the presence of attached portions of the pancreas and locate the ileum by picking up the tip of the caecum and finding the ileum entering the caecum or colon. In cases of enteritis, collect the mesenteric lymph nodes attached to the ileum as they are an excellent source of enteric bacterial pathogens such as *Salmonella* sp., *Mycobacterium paratuberculosis,* and others. Open the colon by laying it out flat and opening several coiled areas. There is generally no need to strip and lay out the full length of the colon in a linear manner. It is important not to open the rectum until completion of the examination the intestines.

22.10.5 Liver, pancreas and duodenum

Nick the duodenal wall near the termination of the bile duct and squeeze the gall bladder gently to determine the patency of the bile duct. Examine the pancreas. Sever the duodenum opposite to the mesentery attachments. Examine the contents and the mucosae. The liver is examined on the surface and several incisions are made across the parenchyma.

22.11 Examination of joints and musculature

Open as many joints as one considers necessary. The carpel, hock, stifle, elbow and shoulder joints can all be examined by opening from the medial side. The right coxofemoral joint has already been opened and examined while reflecting the rear leg. Open the other joints by first reflecting the skin away from the joint to ensure cleanliness. Now locate the area of articulation by moving the joint and incise the medial joint capsule and ligaments. Break open the joint with lateral pressure. Collect joint fluid or swabs from deep in the uncontaminated portion of the joint immediately after opening. It is difficult to physically break open these joints in larger cattle and buffaloes by only incising the medial ligaments and joint capsules. An easier way to open the stifle joints is to cut along the proximal and medial edges of the patella and reflect the patella laterally. Collect fluid or swabs if one observes increased joint fluid or other lesions and then open the joint further. Histological examination of synovial membranes and joint capsule are valuable in confirming a diagnosis of arthritis.

22.12 Examination of the head and brain

For many veterinarians, removing the head and brain is the most difficult part of a necropsy; therefore it is often deleted. Only skin, ligaments, muscle and other connective tissue connect the vertebral column to the head. Therefore, slide the knife in along the vertebral column on both the right and left sides of the atlas (first vertebra) and make incisions laterally through the connective tissues and skin. Gently cut downward over the atlanto-occipital junction until the ventral foramen is exposed.

Proceed to detach the head by inserting the knife into the atlanto-occipital foramen, incising the spinal cord. Then follow the curvature of the occipital condyles with the knife, cutting from the centre laterally and loosening the remaining attachments. Once the atlanto-occipital junction is severed, bend the head backwards and incise the connective tissue, muscle and skin of the dorsal neck.

The brain may be removed by several different methods. In calves (1 to 2 weeks of age), the head may be split longitudinally with a postmortem knife. This is accomplished by inserting the knife vertically just anterior to the brain along the midline and then bringing the handle of the knife downwards toward the base of the skull following the midline as closely as possible. This longitudinal incision is completed by turning the head and using a similar incision to follow the midline along the nasal septa. This is an especially quick and easy method for removing the brain of young calves. A hacksaw or some other type of saw is used to longitudinally split the heads of older animals.

The choice of cutting instrument is often a personal one, however a cleaver is preferred by many. Hatchets may be used, but their relatively light weight results in less skull breakage; therefore, it is necessary to chop the entire incision. An axe is more difficult to control and therefore, is more dangerous to use than either a hatchet or cleaver. When using an axe, hatchet or cleaver protect your eyes from bone chips with safety glasses. The use of a hacksaw or other type of saw is much slower than using a cleaver but is preferred by many because it is more controllable. The cranial landmarks for the incision into the calvaria are at or just behind the eyes. Two side cuts connect the margins of that anterior cut with the dorsal edge of the occipital condyles.

To open the skull using a cleaver, lean the skull backwards, resting it on the ventrum of the occipital condyles. Make the cranial cut straight down. There are two layers of bone to penetrate. Sacrifice the anterior one-quarter to one-half inch of the brain. This cut can be considered deep enough when brain is visible in the depths of the cut. The cleaver will usually stick in the cut and be difficult to retrieve when the cut is deep enough. Lay the head on the side and use restrained bouncing blows to make the two side incisions. Once the side incisions are complete and connected to the cranial cut, reflect the skull caudally by pushing the front edge of the cut skull caudally, leaving the brain in the head.

Nick the meninges with a knife or scissors and slide a swab under the meninges into the region between the cerebellum and cerebrum, if bacterial meningitis is suspected. Completely cut away the dorsal meninges, including the meninges over the cerebellum, before attempting to remove the brain from the skull. If brain removal is attempted with meninges left over the cerebellum, only the cerebrum and a little bit of thalamus will be removed, while the brain stem and cerebellum will be retained in the head. The pons, medulla and cerebellum are all extremely important specimens that are needed for accurate diagnosis and should not be left in the head.

Lean the head backwards and gently support brain. Cut the cranial nerves (begin with the most anterior) and allow the brain to fall out caudally onto a clean surface. After

the brain has been removed, examine the pituitary gland, which will usually remain in the skull.

After removal, incise the brain longitudinally along its midline and lay the incised, flat surface on a clean cutting surface. Then slice one half the brain, making slices no more than ½ to 1 cm thick. The unsliced portion of the brain is used for microbiological examination. In some instances, leave the brain unsliced and submit one half in formalin and one half fresh and chilled in a sterile container to a reference laboratory. If you wish to examine the nasal cavities after examining the brain, cuts across the snout with a saw at the level of the first cheek tooth. If further examination of the mucosa of the turbinate is needed, saw the snout again anterior to the first cut. Alternately, turn the anterior piece of snout and cut it along the ventral midline with a hacksaw or knife trim away the nasal septum to expose the turbinates.

22.13 Removal of the spinal cord

The removal of the spinal cord of a large domestic mammal requires much time and effort, hence it is not performed unless specific clinical signs related to spinal lesions have been observed. If a lesion is localized by clinical examination, it may be necessary to only examine a portion of the spinal cord rather than the entire cord. The method chosen to remove the spinal cord is determined by the suspected lesions, the experience of the veterinarian and available equipment. To remove the cord, split the spinal column along a vertical plane perpendicular to the transverse processes at one edge of the spinal cord. Removal of the spinal cord may then be accomplished without removing the spinal column from the carcass.

22.14 The necropsy report

After the animal has been disposed of, the post mortem report is usually the only remaining permanent trace of the disease/disorder. Unless it is written sufficiently well, the reader, who has not seen the animal, will not get a clear picture of the observed changes and it may be of little value. On the other hand, a well-written report when filed in a practice or in an institution will provide information even many years later, that a newly recognized or defined disease had been in existence in a country for a long time. Such reports also permit the study of changing disease patterns over a period of time. The more immediate advantage of good reports is that, when it is submitted with specimens to the laboratory, it will greatly help in diagnosis. In legal disputes, when confronted with searching questions, the veterinarian may find that a hastily written report, contains only diagnostic descriptions of organs as pneumonic, enteritic, or nephritic, leading to embarrassment to himself and to his client.

Technical details presented in necropsy reports can vary between people. The report is best dictated while the necropsy is being done and edited afterwards, if necessary. Where this is not possible, it should be written immediately after conclusion of the work, when the details are still fresh on one's mind. It should always contain the following information.

- Identification of the animal.
- Abstract of the clinical history.
- Description of pathological findings, with the results of the laboratory examinations.
- Diagnosis, with possible comments on the significance of the various findings and their implications with regard to herd and other problems.

22.14.1 Identification of the animal

This is important for later reference and can be of great value to the farmer and to the veterinarian. Positive identification is an absolute necessity in legal disputes or for insurance purposes. The identity record must include information on species, breed, age, sex, colour and special characteristics such as scars, brand marks and tags, as well as the weight of the animal and its nutritional condition. The time between death of the animal and the necropsy should also be recorded.

22.14.2 Abstract of the clinical history

This should be short but it must contain all the relevant facts, such as the duration of the disease, clinical signs, number of diseased or dead animals, number of other animals in the herd, treatment, and any other significant information including clinical-pathological tests and the clinical diagnosis.

22.14.3 Description of pathological findings

This description is the most important part of the report, and one that often causes difficulties to many persons. The account should begin with a description of the nutritional status of the animal and may include words such as obese, good, moderately good, poor or emaciated. The descriptions have to be objective and diagnostic terms like pneumonia or enteritis should be carefully avoided, as they are sometimes difficult to make only on gross examination. Descriptions of organs or lesions should be word-pictures that will give a person who was not present at the necropsy an exact image of the observed tissues.

With most lesions, the sequence of the description should be the size, shape, surface, colour, consistency and cut surface. Measurements should be given in metric units. If a measuring device is not available, a rough estimate should be made. Sometimes comparative estimates are made relating to common items, especially food items (eg. size of an egg or orange) these can sometimes be confusing. Weighing of organs or other structures is useful.

Terms often used to describe shape, include spherical, ovoid, mushroom-shaped, cylindrical, dome-shaped, nodular, cauliflower-like, circular or elliptical. When describing the shape of a lesion, also note the nature of its border, whether distinct or diffuse, serrated, irregular or regular. Terms like smooth, granulated, nodular, ulcerated, eroded, pitted, raised, depressed, glistening, scaly, hairy, transparent, moist, dry, fibrinous are used to describe surfaces and to some degree to cut surfaces. When describing colours, it is preferable to use the names of common colours and if

necessary, specify further using terms such as dark or light. Consistency may be hard, firm, soft, gelatinous, mucoid, caseous, cream-like, crepitant, adhesive, gritty, granular, or dry.

The order in which the different organs are described varies e.g. system based or in the order they are revealed at necropsy. The latter has an advantage in that recording could be made while the necropsy is in progress, therefore one is less likely to forget a description of an organ or system. Moreover, a person reading the report is able to follow the necropsy step by step. When writing the report on an animal which has been systematically examined, it should not be necessary to mention that certain organs are normal, or show no significant changes, unless one wishes to stress the fact. This would be the case when lesions in these organs could be expected from the clinical history or clinical diagnosis, or from certain experimental procedures, but are not present. Excessively shorten forms of description may lead to misinterpretation therefore it is better to write full sentences and use the present tense.

22.14.4 Diagnosis

The diagnosis is based on the observed lesions, taking into account available laboratory tests and clinical history. After completion of the necropsy, the diagnosis arrived at may be only tentative, subject to the results of further laboratory examinations. One should strive to arrive at an aetiological diagnosis, giving the cause of the disease. If this is not possible, a morphological diagnosis (bronchopneumonia, mucopurulent, acute, severe, locally extensive) is the next best and may be of great value in the examination of similar cases. Unless the diagnosis is self-explanatory, it should be followed by a brief description of the significance of the various findings.

22.14.5 Sampling for laboratory investigations

The collection of samples for microbial culture should always be given priority with a view to reducing contamination to a minimum. Ectoparasites such as ticks, mites and lice should be sent dry for parasitological examination. Alimentary contents and washings should be sent fresh but if there is likely to be a delay, add 40% formaldehyde to bring to a final concentration of 5%, or submit in the frozen state.

Samples for histological examination should be collected from representative areas. A knife or scalpel is preferred to scissors when cutting tissue samples for histology. Samples should generally be about 1 cm thick. Avoid rubbing mucosal surfaces. The selected samples should be fixed immediately on collection in a wide-mouthed bottle. The fixative could be 10% formalin or formol-saline. Commercially available formalin is 40% formaldehyde and a 10 per cent solution can be prepared by mixing one part with 9 parts of water. To prepare formol-saline, add 8.5 g of sodium chloride per litre of 10% formalin. Formol-saline is osmotically preferable to formalin. The injection or infusion of fixative into a hollow organ before opening can reduce the damage to the delicate mucosa imposed by handling. The ratio between the tissue and fixative should be around 1:20.

Selected references

Carlton, A.W. and McGavin, M.D. (1995) *Thomson's Special Veterinary Pathology*. (2nd Edition). Mosby, St Louis, USA..

Cheville, N.F. (1988) *Introduction to Veterinary Pathology*. Iowa State University Press. USA.

Kelly, D.F., Lucke, V.M. and Gaskell, C.J. (1982) *Notes on Pathology for Small Animal Clinicians*. Wright, P.S.G., Bristol, UK.

Strafuss, A.C (1987) *Necropsy – Procedures and Basic Diagnostic Methods for Practising Veterinarians*. Charles Thomas Publishers, Illinois, USA.

CHAPTER 23

Pharmacological Basis of Therapeutics

Preeni Abeynayake

Introduction

The availability of an increasingly large variety of potent drugs demands that veterinarians should critically examine their approach to drug therapy. Since there is no absolutely 'safe' drug, the art of therapeutics requires consideration to ensure the optimum use of the chemical for the well-being of the patient.

Rational therapeutic regimen entails choice of

- the right drug (therapeutic indications)
- to the right patient (avoid contraindications)
- at an appropriate dose
- by an appropriate route
- at recommended frequency
- for the optimum duration.

Prerequisites to rational therapy include

- a proper diagnosis
- understanding of the
  - pathophysiology of disease
  - pharmacology of the drug and
- establishment of therapeutic objectives.

23.1 Pharmacodynamics

The process of modification of existing biochemical or physiological actions are referred to as pharmacodynamic activity of drugs and this results in a therapeutic response. This modifying process can be done by several mechanisms. The majority of therapeutic agents alter cellular or organ functions through an interaction with cellular components or contents as shown below.

Drug + Receptor ↔ D - R complex → Response
* therapeutic effects
* unwanted (side effects) effects
* toxic effects

With respect to the host, chemotherapeutic agents have an indirect action. They allow the host to recover by killing the causative agent (i.e. bacteria, fungi, parasites). On the other hand, replacement therapy (i.e. fluid, electrolytes, blood, hormones, vitamins and nutrients) allows recovery by supplying nutrients in pure and easily utilizable form.

In certain situations, the action depends on the physio-chemical properties of the drug as indicated below.

- *Volatile anaesthetics* penetrate the alveolar mucosae to be transported into the central nervous system by virtue of their lipid solubility.
- Strong acids and alkalis produce *caustic* effects through cell death and lysis.
- *Astringents* precipitate surface proteins of gut mucosae to form a protective layer.
- Non-absorbable solids given orally act as *adsorbents*, having a binding ability with toxins whereby the toxicants become biologically unavailable.
- *Lubricants* ensure the easy passage of diagnostic instruments.
- *Osmotically active agents* retain water and may bring about a purgative action or diuresis.

23.2 Pharmacokinetics

Pharmacokinetics means the time course of the drug effect, and is described as dissolution, absorption, distribution, biotransformation and excretion of therapeutic agents.

23.2.1 Dissolution

It is a rate-limiting step that determines the release of a drug from its dosage-form. It frequently controls the rate of drug absorption. The dissolution process can be controlled by administering the drug in different salt forms, as seen in different salts of benzyl penicillin.

e.g. (i) sodium salt of benzyl penicillin is a water soluble form and therefore has a short duration of action and high rate of excretion.

(ii) procaine salt of benzyl penicillin makes it water insoluble and thereby the duration of action is maintained over a period of 24 hours.

(iii) benzathine salt of benzyl penicillin being the most insoluble salt of benzyl penicillin has longer duration, since it is slowly released from the site of administration.

23.2.2 Absorption

After the drug is released, the rate of absorption is determined by several factors. The route of administration is the major determinant of the speed of therapeutic effect. The intravenous route provides an almost immediate response with a predictable

concentration in plasma. The rate of absorption from parentral sites of administration depends on the vascularity of the injection site. For the majority of orally administered drugs, the small intestine is the principal site of absorption, as it has an extensive surface area with a rich blood supply. However, irrespective to the route of administration, the degree of ionization (expressed as the dissociation constant of the drug: pK_a) and the pH difference across the cell membranes, determine the ability of drug penetration across the cell membrane. It can be concluded that only the lipid soluble, non-ionized moiety of organic electrolyte that is free and unbound in plasma can penetrate the cell membrane.

23.2.3 Distribution

After a drug is absorbed or injected into the blood stream, it may be distributed into interstitial and cellular fluids. The pattern and extent of distribution reflects certain physiochemical properties of drugs and the regional blood flow. Heart, liver, kidney, brain and other well perfused organs receive most of the drug within the first few minutes. Delivery of a drug to muscle, most viscera, skin and fat is slower and may require several minutes to several hours to reach a steady state.

Distribution also may be limited by drug binding to plasma proteins, particularly albumin-binding for acidic drugs and α_1-acid glycoprotein binding for basic drugs. Extensively and strongly plasma protein bound drugs have limited access to cellular sites of action, while they are metabolized and eliminated slowly. Drugs may accumulate in tissues in higher concentrations than would be expected, as a result of pH gradients (ion trapping), binding to tissue receptors or partitioning into lipid.

23.2.4 Biotransformation

The lipophilic drugs can get reabsorbed through renal tubules and therefore may persists in the body without being excreted. The biotransformation of drugs into more hydrophilic metabolites is therefore essential for the termination of the biological activity of lipid soluble drugs before renal elimination. Drug biotransformation reactions are classified as either *Phase I* reactions or *Phase II* biosynthetic reactions.

Phase I reactions: These generally result in a loss of pharmacological activity but occasionally result in modified pharmacological activity. Further, inactive prodrugs are converted rapidly to biologically active metabolites, often by hydrolysis.

Phase II reactions : During phase II conjugation, resultant metabolites of phase I reactions, get combined with endogenous compounds to form highly water soluble conjugates. These endogenous compounds can be glucuronic acid, sulphate, glutathione, amino acids or acetate. The resultant highly polar conjugates are generally inactive, nontoxic and are rapidly excreted in the urine and faeces.

The rate and extent of biotransformation may be modified in the following situations.

Availability of metabolizing enzymes is a *genetic factor* and can be accounted for species and breed variations of drug metabolism.

Enzyme availability varies with the *age* of an individual. Neonates and geriatric patients are the poor metabolizers.

The *disease condition* of the animal; particularly conditions affecting the liver may lead to reduced rate of metabolism.

- *Concurrently administered* drugs may influence the rate of metabolism of each other, if they are capable of inducing or inhibiting liver enzymes.

These factors may account for unexpected decreased efficacy, prolonged pharmacological effects or increased toxicity.

23.2.5 Excretion

Drugs are eliminated from the body either unchanged or as metabolites after liver biotransformation. Water soluble polar compounds are efficiently eliminated *via* all routes except through the lungs. Lipid soluble drugs need to be metabolized to more polar compounds for elimination. Orally ingested, unabsorbed drugs are mainly eliminated in faeces. Drug metabolites excreted in bile may follow the faecal route of excretion. Excretion of drugs or their metabolites in milk is important because of the potential hazard to consumers. Pulmonary excretion is important for anesthetic gases and vapours. The kidney is the most important organ for elimination of water soluble drugs or water soluble metabolites of lipid soluble drugs. Renal excretion involves three processes, glomerular filtration, active tubular secretion and passive tubular reabsorption.

The amount of *glomerular filtration* of a drug or its metabolite is dependent on the extent of plasma protein binding as well as the glomerular blood flow. In the proximal convoluted tubule, certain organic anions and cations are added to the glomerular filtrate by active, carrier mediated *tubular secretion.* In the proximal and distal tubules, the non-ionized form of weak acids and bases undergo *passive reabsorption.* When the tubular urine is made more alkaline, weak acids are excreted more rapidly, primarily because they are more ionized and possible reabsorption is decreased. When the tubular urine is made more acidic, the excretion of weak acids is reduced. Alkalinization and acidification of urine have opposite affects on excretion of weak bases. This theory is applied in the treatment of drug poisoning, to hasten the rate of elimination of the poison. Alteration of urinary pH may result in a significant change to drug elimination depending on the pK_a value of the chemical.

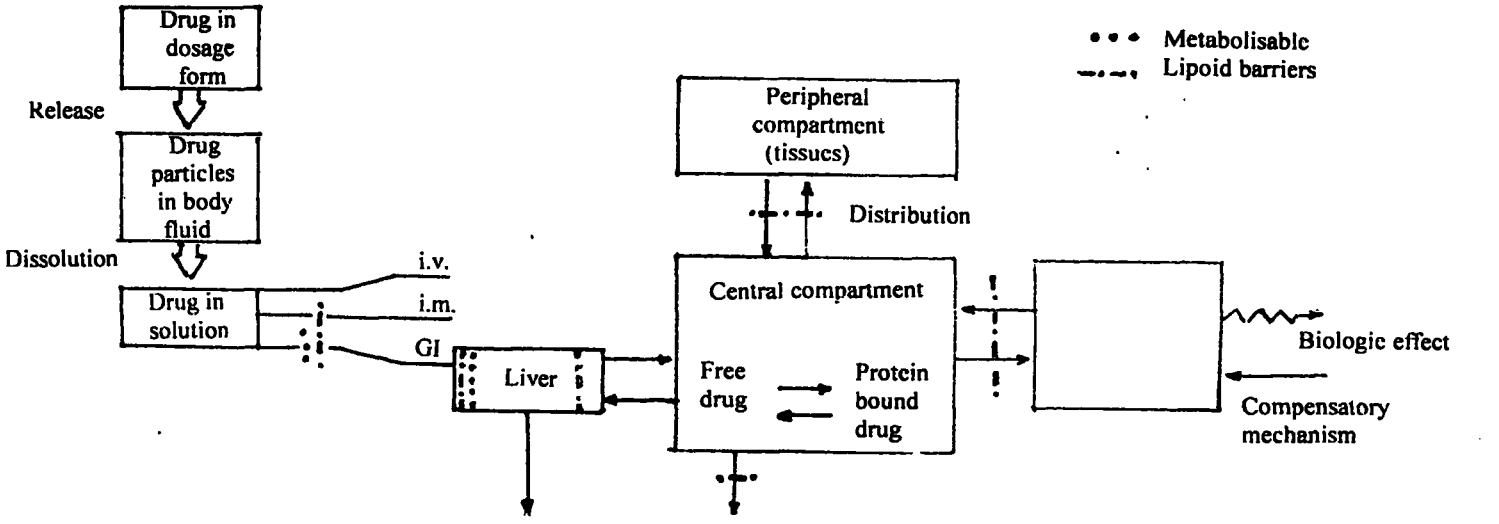

Fig..23.1 Factors influencing the onset, duration and intensity of drug effects *(Adapted from Barr, W.H., 1968)*

The serum drug concentration profile is an integrated picture of the processes affecting drug distribution and elimination in the body. The observed concentration at any time is thus simply a sum of the distribution and elimination contributions. There are several factors influencing the time-course of the drug effect as shown in the Fig 23.1.

The treatment regimen should be designed to achieve maximum effectiveness, minimum host toxicity without violative residues in edible tissues (Fig. 23.2). This is fairly difficult to achieve with a specified dose given at a fixed frequency, due to the fact that clinically presented animals are of differing ages, gender, body weight and nutritional status. Moreover, the disease being treated often alters the disposition of the drug. The current trend is to approve doses expressed as a range rather than as a specified dose. This approved range is referred to as the *therapeutic window* of the drug (e.g. 5 -10 mg/kg twice daily).

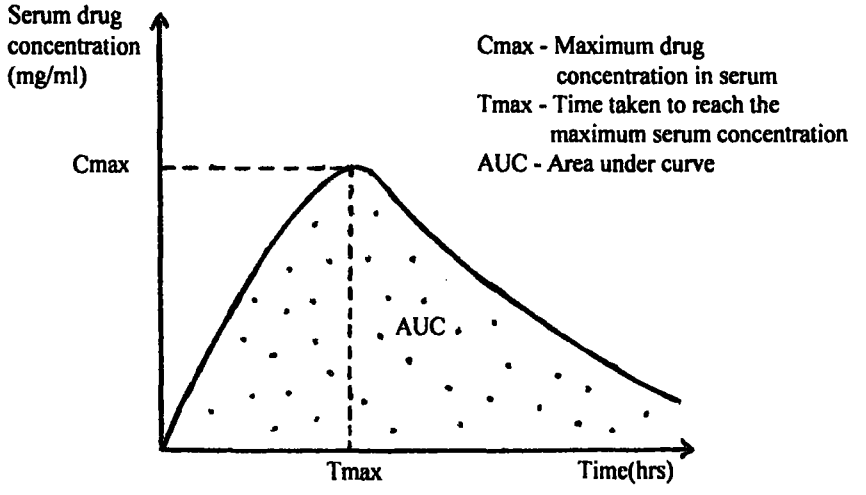

Fig. 23.2 Serum drug concentration against time, after single administration at any extravascular site.

The rational treatment regimen enables one to achieve the effective drug concentration at the target site, after administration by different routes. This can be measured in terms of *bioavailability*, which is the amount of the drug that reaches the systemic circulation.

Bioavailability not only provides a guidance to the therapeutic regimen, it is a useful parameter which can be used in comparing different pharmaceutical products containing the same active ingredient. Factors that can influence bioavailbility are

- rate of administration
- incomplete dissolution

- incomplete absorption
- metabolism in the gut and gut wall
- metabolism in the liver.

When the bioavailability is similar in products which contain the same active ingredient, such products are considered as *bioequivalent.*

In a multiple dose therapeutic regimen, when the rate of drug absorption equals the rate of drug elimination, then a steady state concentration (C_{SS}) will be achieved in the general circulation.

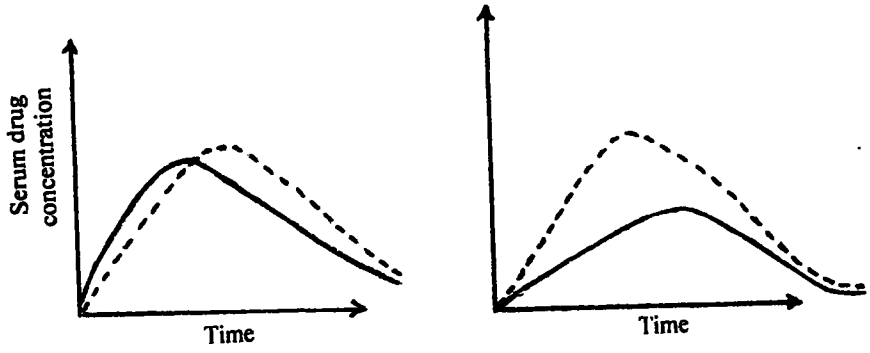

Fig. 23.3(a) Fig. 23.3(b)

Fig. 23.3 Serum profiles of two products of the same active ingredient where (a) bioavailability is similar and (b) bioavailability is significantly different.

The steady state concentration is possible due to

- multiple doses given at regular intervals (Fig. 23.4)
- loading dose followed by maintenance doses at regular intervals
- constant rate infusion
- sustained release devices.

Fluctuations in drug concentration should be within the therapeutic range. Therefore the *therapeutic objective* is to provide a sufficient amount of the drug at appropriate intervals to maintain concentrations greater than the minimum effective concentration but less than those that produce toxic effects. Toxic effects may occur when a drug accumulates to reach toxic concentrations, as when the drug is given in excessive doses or too frequently.

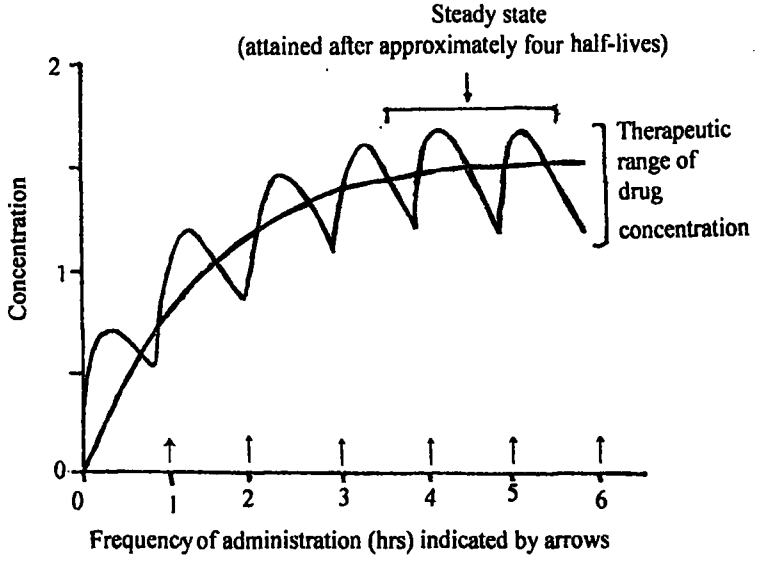

Fig 23.4 Pharmacokinetic relationship for repeated administration of drugs

If pharmaceutical or biological products are used within the *label recommendations*, the responses can produce either therapeutic or unwanted effects. If large amounts, well above the label recommendations are used, the resulting responses will be toxic reactions.

23.3 Step-by-step guide to rational therapy

The rational approach for treating diseases can be assisted by the application of the Minimal Essential Drug Information Check list (MEDIC) developed by Coppoc and Stuckey (1977). Accordingly, the steps involved in making the a rational decision in the treatment of a disease is presented as follows.

23.3.1 Therapeutic objective

This is the specific pathological process that gets altered with the use of the drug. An accurate diagnosis and a clear knowledge of the drug action is a pre-requisite to arrive at a therapeutic objective.

23.3.2 Route of administration

This means the selection of the best route for drug administration. The aim is to achieve maximum drug concentration at the target site while minimising side effects. For localised lesions, local applications (orals, pour-ons, udder infusions, intrauterine administrations, subconjunctival injections etc.) are preferred to systemic administrations. Intravenous injections give immediate high concentrations but accompanies the risk of toxicity. Administration by intramuscular and subcutaneous routes gives moderately high whilst longer durations of activity but lesions and residues at injection sites cannot be ignored. During treatment of a herd or a flock, optimum efficacy can be achieved economically by combining different routes within a single therapeutic regime, e.g. for treatment of pinkeye in cattle, subconjunctival injections of antibiotics may be followed by local instillations of eye ointments.

23.3.3 Product dosage form

To suit the selected therapeutic regime the drug should be available in the required dosage form, since each product is specific for the prescribed route indicated on the label.

23.3.4 Dosage

Generally, the recommended dose for the specific therapeutic indication should be used and this is indicated on the product label. There can be situations where the dose has to be modified as a precautionary measure, if indicated on the label or drug-insert. To assist the clinician in such situations, a range is suggested which is referred to as the "therapeutic window" of the product.

23.3.5 Dosage interval/frequency of administration

The aim is to maintain the drug concentration within the therapeutic range at the target site. With too frequent administrations, the drug may accumulate to reach toxic levels while low frequencies will maintain ineffective concentrations at the target site. Several dosage forms of the same drug may be used by different prescribed routes to maintain the steady state of drug concentration.

23.3.6 Duration of therapy

The treatment regime should be continued for a sufficiently long duration for a complete cure without causing problems. For example, the use of antimicrobial agents for unnecessarily long periods will not only disturb the normal flora but may promote the emergence of resistant organisms.

23.3.7 Withdrawal period

If a drug is approved for food-producing animals, instructions relating to the withdrawal period given on the label must be observed. If the product is used outside the label recommendations, withdrawal periods (by default) should be as follows (Bishop, 1996):

- meat - 28 days
- milk - 7 days
- eggs - 7 days
- fish - 500 degree days (mean daily temperature ^{0}C x total number of days; e.g. at 10 ^{0}C for 50 days is 500 degree days)

23.3.8 Cost of treatment

This should include the clinician's time, cost of dispensers and devices, cost of drugs and others. The cost of treatment should not exceed the desire of the owner to pay. The cost should be appropriate to the seriousness of the disease.

23.3.9 Special precautions

There can be special precautions that must be observed to enhance the effectiveness or safety of a drug. For example, drugs which will depend on renal elimination tend to remain longer in animals with renal problems. Drugs that are metabolised in the liver prior to elimination, tend to accumulate, if administered to animals with liver impairment. Moreover, drugs may interact with other drugs given at the same time. In such situations, a warning is usually given in the drug packaging under "Directions of use" panel.

23.3.10 Drug contraindications

Conditions for which the drug should not be administered need to be considered. For example, in lactating animals consideration should be given to residues in milk. Milk residues may not only be harmful to the human consumer, but also be toxic to the new born calf. Secondly, special care has to be taken with drugs administered during pregnancy. Certain drugs not only damage the conceptus but may terminate the pregnancy.

23.3.11 Adverse reactions

With certain drugs, adverse reactions are possible at the recommended dosage schedule. Some of these are to be expected, if such reactions can be explained by the known pharmacology of the chemicals. Some other reactions may be unexpected and cannot be explained with the present knowledge of the pharmacology of the drug. It is good to detect or predict potentially serious reactions before they endanger life.

Reporting suspected adverse drug reactions or inefficacies are a recommended practice (form is annexed).

23.3.12 Antidote

In the event of an adverse reaction, the animal has to be treated in order to minimise the danger. For certain therapeutic agents, specific antidotes are available (e.g. yohimbine against xylazine). Otherwise symptomatic treatment can be undertaken. When using potentially hazardous drugs, antidotes, emergency drugs and equipment should be readily available.

23.3.13 Therapeutic evaluation

The effectiveness of the treatment has to be evaluated using reliable parameters. The selected parameters should well display the therapeutic response or a clinical improvement of the treated animal.

23.3.14 Follow-up procedure

There should be a follow-up procedure either by a revisit by the client, telephone call or by visiting the farm. This will confirm the success of the therapeutic regime.

23.4 Species variation observed with veterinary drugs

Veterinary pharmacology is a difficult but a challenging discipline to master because of the multiplicity of species considered. These species differences are the essence of the subject as distinguished from medical pharmacology. The species variation observed for the drug effect can be considered in two phases. The reasons for differences in the pharmacokinetic phase can be due to

differences in dietary habits that effect orally administered drugs.
pH differences in different body compartments which influence drug penetration across the membrane and thereafter distribution and excretion.
differences in the binding intensity or capacity of receptors located in plasma proteins that influence distribution.
differences in the availability of liver enzymes that influence biotransformation and thereafter excretion.

Differences observed in the pharmacodynamic phase can be due to

differences in receptor distribution and their binding intensity, which in turn influence the inherent drug efficacy.

It is conclusive that drugs used in treatment are potential toxicants and have to be used with a definite justification, to suit each disease condition in the species

recommended.

23.5 Extra-label uses

A large number of preparations are registered and labeled for the prevention and treatment of diseases in domestic animals. Still, there are many conditions for which either no approved preparation is available, or one is required to deviate from the registered conditions. In such situations, clinicians are compelled to use products outside the label recommendations and these situations are referred to as extra-label or off-label uses of drugs. Because of the professional training veterinarians have received, they are competent to take the responsibility involved in the extra-label use of pharmaceuticals.

To ensure that the rational choice of therapy is continued there are some additional factors that have to be highlighted. Safety of selected treatment regimen could be considered and extended to the

- target species being treated
- the person administering the preparation
- the environment
- the consumers of animal products

Before commencing the treatment, the proposed regimen should be explained to the farmer and his consent has to be obtained for the extra label use of pharmaceuticals. Where necessary, farmers should be made aware of the importance of withholding periods before drugs are recommended to farmers, in order to ensure that consumer food commodities are free of violative drug residues.

Drug information pertaining to residues in food animals are available in several forms where label information is absent, as in extra - label application. The Food Animal Residue Avoidance Databank (FARAD) is a USDA approved electronic databank of scientific and regulatory information, that can assist the veterinarian, producer or other individuals in making rational choices in preventing drug residues in food animals.

Selected references

Coppoc G.L. and Stuckey W. J. (1977) *J. Veterinary Medical Education* 4, 171

Craigmill A.L., Sundolf S.F. and Riviere J.E. (1994) *Handbook of Comparative Pharmacokinetics and Residues of Veterinary Therapeutic Drugs.* CRC Press.

Riviere J.E. (1991) *Handbook of Comparative Pharmacokinetics and Residues of Veterinary Antimicrobials.* CRC Press.

Yolande Bishop (1998) *The Veterinary Formulary* (4th edition), British Veterinary Association.

Annexure

Report on Suspected Adverse Reactions and Inefficacies of Products Used on Animals

Underline the most appropriate designation

SUBMITTER

Veterinarian Manufacturer Farmer or Animal Owner Other

Veterinarian Manufacturer Farmer or Animal Owner Name..... Address..... Telephone..... Fax.....	Other Date of Submission .....

DETAILS OF ANIMAL OWNER

Name.....
Address.....
Telephone..... Fax.....

THIS REPORT IS ON

Adverse reactions Lack of effect Adverse reactions on users

PRODUCT DETAILS

Product Name .....
Active constituent(s) .....
Manufacturer/Registrant .....
Storage conditions of the product at the time of administration .....

Batch No: .....
Expiry date: .....

ANIMAL DETAILS

Species .....
Breed .....
Sex .....
Age .....

Number treated .....
Number affected .....
Number dead .....
Estimated weight .....

DISEASE BEING TREATED

History and clinical signs before treatment.....
.....
.....

Diagnosis.....
Environmental conditions prior to/at initial treatment. (eg. weather, pasture conditions, water availability).....

TREATMENT DETAILS

Dose rate	Date & time of treatment .....
Route	Date & time when symptoms were first noticed .....
Frequency	Date & time of death or recovery.....
Duration	.....

Any other drugs at the same time .....
.....
.....

REACTION DETAILS

Description of clinical signs following treatment.....
.....

Subsequent treatment undertaken and outcome.....
.....
.....
Results of laboratory tests and post mortem .....
.....

This report to be posted to

Dr. P. Abeynayake
Dept. of Paraclinical Studies
Faculty of Vet. Med. & Animal Science
University of Peradeniya
Tel: 08-389542 Fax: 08-388205

FOR OFFICE USE ONLY

Date received:.....
Category of reaction:.....
Decision:.....

CHAPTER 24

Prescribing Antimicrobial Drugs for Ruminants

Preeni Abeynayake

24.1 General considerations

Since cattle, buffaloes, sheep and goats are productive animals, the prescriber should ensure that market products are free from chemical residues. The farmer should be advised to adhere to withdrawal periods (WP) stated on product labels for different consumer commodities (e.g. milk, meat and fibre). If no WP is given in labels, or in extra-label enclosures, the standard WP should be applied, while the prescribing veterinary clinician holds the responsibility.

The use of certain drugs are restricted for treatment of food producing animals, in order to safeguard the consumer. For example, the use of chloramphenicol is limited to situations where no other suitable antibacterial agent is available (Bishop, 1996). This is because the presence of chloramphenicol residues in human food may lead to the development of bacterial resistance by way of resistant bacteria entering the food chain *via* carcass contamination. This restriction of use has been introduced because chloramphenicol is an effective antibiotic for treatment of human typhoid at present and, therefore it is necessary to maintain its valuable efficacy by minimising the chances of the emergence of bacterial resistance. Moreover chloramphenicol residues have given rise to an idiosyncratic reaction in man, where extreme sensitivity to very low amounts of the drug has been observed. In summary, consumer safety is an important aspect that has to be kept in focus while treating ruminants reared as food producing animals.

Drugs may be prescribed for treatment of an individual animal or in group medication. In the latter situations, administration *via* feed or drinking water is preferable where possible. Individual animal treatment may be performed as a corrective measure when one animal is diseased or on a herd-basis during prophylaxis or metaphylaxis programmes.

For parenteral administration, injections may be given subcutaneously (SC), intramuscularly (IM), intravenously (IV) or intraperitonealy (IP). The neck region is the preferred site for IM or SC injections, as this will prevent damage to valuable meat-cuts and also reduce the occurrence of injection-site lesions, thereby residue violations (Fig. 24.1). Not more than 20 ml should be administered at any single site, if given by the IM route.

Slow release devices are available, which reduce the frequency of administrations, thereby minimising the associated expenses (i.e. labour cost, time spent). These devices provide continuous or pulsatile release of the drug over a few weeks. Ruminant boluses (dosage forms to be retained in the rumen) should not be given to cattle weighing less than 100 kg body weight or are less than three months of age as they do not have a functional rumen. Operators should be trained in the method of administering these boli.

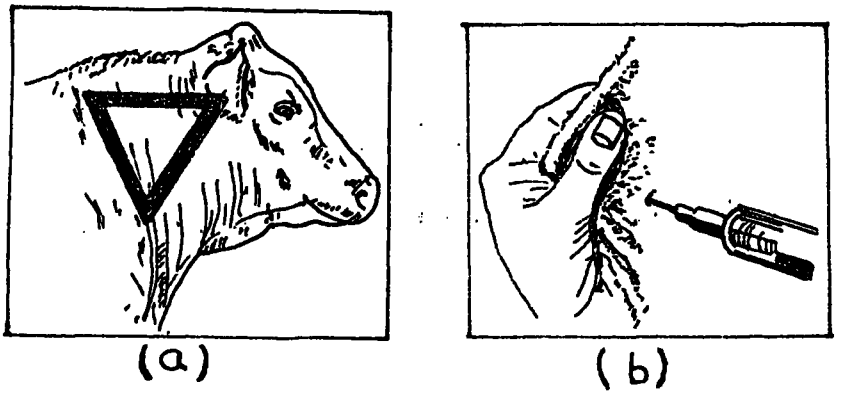

Fig. 24.1 Diagrammatic presentation of (a) proper site and (b) proper technique of IM or SC injections.

24.2 Adverse drug reactions related to antibacterial agents

Orally administered antibacterial drugs may disturb bacterial fermentation processes in the rumen, resulting in digestive disturbances or drug inactivation. The strict adherence to the label recommendations (where possible) will reduce some of these expected adverse reactions.

In cattle, rapid IV administration of tetracyclines may cause cardiovascular collapse due to chelation of calcium ions in heart muscle, with consequent vasodilation and myocardial depression.

24.3 Antibacterial drugs

Selection of a suitable drug: Antibacterial drugs are often misused when they are clearly contra-indicated in many instances. However, when antibacterial therapy is essential, a justifiable therapeutic decision has to be taken. The basic objective is to maintain an effective drug concentration at the site of infection, for the optimum duration. The effective concentration, which should be well above the minimum - inhibitory - concentration (MIC) is dependent on the susceptibility of micro-organisms and the pharmacokinetics of the drug. An accurate clinical diagnosis, identification of the implicated organisms with their susceptibility is complementary for rational chemotherapy. The selected antibacterial agent should be available in suitable dosage-forms to be used by the clinician. The duration of therapy is usually 5 - 7 days except in special circumstances.

24.3.1 Benzyl penicillins

They are effective on Gram-positive aerobes, *Clostridium, Bacteroids* spp. fastidious Gram-negative aerobes such as *Haemophilus, Pasteurella, Leptospira* and some *Actinobacillus* spps. Benzyl penicillin is available as a range of salts that differ in their solubility and thereby the duration of action.

24.3.1.1 *Sodium Benzyl Penicillin*: This is a highly water soluble preparation and therefore the effective concentration is maintained for not more than four hours.

(a) Contra-indications: Use in small herbivors (eg. guinea pigs, hamsters or rabbits).

(b) Warning: All penicillins and cephalosporins may cause hypersensitivity following administration to any host species. These adverse reactions may be clinically manifest as a Type I hypersensitivity reaction with a varying clinical picture, depending on the target species and the route of exposure. Handlers with history of allergy should not get exposed to penicillin products.

(a) Dose: cattle/buffalo - 10mg/kg, IV twice daily.

24.3.1.2 *Procaine penicillin (procaine benzyl penicillin)*

(a) Dose: cattle/buffalo - 10-15mg/kg IM or SC, once daily.
(b) WP: for milking animals 3 days, for meat animals 4 days.

24.3.1.3 *Procaine penicillin and benzathine penicillin combination*

The strengths of procaine penicillin and benzathine penicillin are 150 mg/ml, respectively.

(a) Dose: 0.04 ml / kg IM, repeat after 3-4 days, if required.
(b) WP: meat animals 14 days, milking animals 3 days.

24.3.2 Ampicillin and Amoxycillin

These are broad spectrum penicillins which have slightly less activity than benzyl penicillin against Gram-positive bacteria and anaerobes. They exert a considerably greater activity against Gram-negative bacteria although the activity is poor against *Klebsiella*, some *Proteus* spps. and *Pseudomonas* spps. Ampicillin and amoxyllin are susceptible to beta-lacamases, produced by *staphylococcus* spps. and Gram-negative organisms such as *E. coli* and *Haemophilus* spps. But this could be overcome by combining with a beta-lactamase inhibitor. Clavulanic acid is a potent beta-lactamase inhibitor which is usually combined with amoxycillin to be effective against most strains of *Staphylococcus aureus*, some *E. coli* as well as *Bacteroids* and *Klebsiella* spps.

Ampicillin should be given to fasting animals, or at least an hour after meals for better absorption by the oral route. Amoxycillin is less affected in the presence of food and therefore is better absorbed than ampicillin. Both are excreted in bile and urine. Depot preparations are available for both agents to prolong the action. Depot oil-based ampicillin preparations contain aluminium monostearate in the formulation.

Contraindications: Oral administration to young animals with functioning rumen.

24.3.2.1 *Ampicillin*

(a) Dose: calves - oral 10 - 15 mg/kg twice daily
- IM or SC injections 2-7mg/kg once daily
(WP: milking animals 1 day and meat animals 21 days)
- Depot IM injection 15mg/kg repeat after 2 days
(WP: milking animals 1 day, meat animals 21 days)

24.3.2.2 *Amoxycillin*

(B) Dose: calves - oral 8mg/kg twice daily
- IM injections 7mg/kg daily
(WP: milking animals 2 days, meat animals 21 days)
- Depot IM injections 15mg/kg repeat after 2 days
(WP: milking animals 3 days, meat animals 21 days)

24.3.2.3 *Amoxycillin (as trihydrate) and Clavulanic acid*

(a) *Dose*: calves - oral 5-10mg/kg twice daily
- SC & IM injections 7 mg/kg daily

(b) WP : milking animals 1 day, meat animals 14 days

24.4 Cephalosporins

Cephalosporins are closely related to penicillins but relatively nontoxic and less likely to cause allergic reactions. Suitable for use in laboratory animals such as rabbits and rodents.

The first generation cephalosporins (e.g. cephalexin) are active against a range of both gram-positive and gram-negative organisms including beta-lactamase producing *Staphylococci* spps. However, *Pseudomonas* and many *proteus* spp. are resistant. They are given only by the oral route. Successive generations are characterised by being less absorbable following oral administration.

The second generation (e.g. cefuroxime) is active against some Gram-negative organisms which are resistant to the first generation and has greater activity against *Actinobacillus* (*Haemophilus*) spp.

The third generation (e.g .ceftiofur, ceftazidine and cefoperazone) have good Gram-negative activity (including *Pseudomonas* spps). They retain activity against Streptococcus spp. but have poor activity against Staphylococcus spp.
The fourth generation (e.g. cefquinome) continues the same trend.

24.5 Tetracyclines

The tetracyclines are broad-spectrum bacteriostatic antibacterials, active against bacteria, *Mycoplasma*, *Chlamydia* and *Rickettsia*. They are of little value against *E. coli*, *Salmonella*, *Proteus* and *Pseudomonas* species. Acquired resistance is now widespread among bacteria, and resistant isolates of *Pasteurella multocida* have been

reported (Abeynayake *et al.*, 1992).

Tetracyclines may be irritant when given by the intramuscular route unless they are formulated with polyvinylpyrrolidone as a vehicle. Depot preparations will maintain effective plasma concentrations for 72-96 hours. The same preparations may be given intravenously, but rapid injection by this route may cause cardiovascular collapse, apparently due to chelation of calcium.

Oral administration to calves may cause diarrhoea. Absorption of tetracyclines from the gut is variable and is reduced in the presence of milk (except doxycycline), antacids and by calcium, iron, magnesium and zinc salts. Horses that are given parenteral tetracyclines and thereafter exposed to stress may suffer from severe enterocolitis, which can prove fatal.

The main excretory routes for tetracyclines are the urinary system and the gastro-intestinal tract *via* the biliary system. Tetracyclines undergo enterohepatic cycling, which may explain the emerging acquired resistance to this group of antibiotics. A newer product of the tetracycline group, doxycycline has more advantages than older tetracyclines. It is more lipophillic, therefore gut absorption is better and is less affected by the presence of milk and calcium salts. Doxycyclines penetrate well notably the lung and CSF. Since they are excreted through bile, they are liable to produce enterocolitis in the horse.

24.5.1 Chlortetracycline

(a) *Contraindications*:
- Oral administration to ruminants with functional rumen.
- during renal impairment
- last 2 - 3 weeks of gestation and upto 4 weeks of age in neonates
- during dysphagia
- diseases accompanied by vomiting

(b) *Side affects*: may cause vomiting and diarrhoea

Dose: calves 1-20 mg/kg BW

24.5.2 Oxytetracycline

Dose: Cattle, sheep and goats

Short-acting preparation - IM or IV injections, 2-10mg/kg. daily.

Long acting preparations - Depot IM injections, 20mg/kg repeat after 2-4 days or 30mg/kg repeat after 6 days.

Calves - oral 10-30 mg/kg twice daily.

24.6 Aminoglycosides

Aminoglycosides are bactericidal antibiotics active against gram-negative and gram positive organisms, but not against *Streptococci.* Only gentamicin is active against *Pseudomonas aeruginasa.* Aminoglycosides are taken up into bacteria by an oxygen-dependent process and are therefore inactive against anaerobic bacteria. They are more active in alkaline media, which is of particular importance when treating urinary infections. Aminogylcosides are synergistic with beta-lactam antibiotics. Acquired resistance exists. Enteric bacteria may gain the ability to produce a range of aminoglycoside-inactivating enzymes particularly if a sub-therapeutic dose is given. Different members of the group vary in their susceptibility to these inactivating enzymes.

The aminoglycosides are not absorbed through the gut, therefore this route is reserved for the treatment of gastro-intestinal infections. After parenteral injections, they are poorly distributed. Since these are water soluble chemicals, elimination is solely by renal or faecal excretion depending on the route of administration. Aminoglycosides have receptor binding sites in the kidney which lead to detectable residue levels for a considerable duration.

The important side-effects are vestibular or auditory toxicity, to a lesser extent nephrotoxicity and impairment of neuromuscular transmission. Risk of toxicity following systemic administration varies with different members of the group. Neomycin is particularly toxic to auditory and renal systems. Streptomycin and dihydrostreptomycin are ototoxic. Gentamicin is ototoxic and nephrotoxic. Simultaneous administration with other potentially ototoxic drugs such as loop diuretics should be avoided. Aminoglycosides are well absorbed from the peritoneal cavity and instillation during surgery may result in drug overdose and transient respiratory paralysis.

24.7 Macrolides and Lincosamides

The macrolides include erythromycin, spiramycin, tiamulin, tilmicosin and tylosin, while clindamycin and lincomycin belong to the related lincosamide group. All are basic compounds that are well absorbed following oral administration, re-inactivated by hepatic metabolism. Due to the basic nature, they are concentrated by ion-trapping in acidic fluids such as milk (requiring prolonged withdrawal periods following systemic injections) and prostatic fluid. They are bacteriostatic in action.

Tylosin has good activity against Mycoplasma spps. and a number of Gram-positive aerobes, but little activity against Gram-negative organisms and anaerobes.

Tiamulin has a broader spectrum of action, which includes more fastidious Gram-negative organisms such as *Haemophilus*, *Bordetella*, *Pasteurella* and *Actinobacillus* spp. and also a number of anaerobic organisms. Tiamulin is known to cause growth retardation when administered with monensin, narasin or salinomycin.
Erythromycin is active against *Streptococci, Staphylococcus aureus* including penicillin resistant strains, the more fastidious Gram-negative bacteria and anaerobes.

It is likely to be the drug of choice against *Compylobacter*. Erythromycin has less activity than tiamulin or tylosin against *Mycoplasma* spp.

Spiramycin is combined with metronidazole in the treatment of mixed aerobic/anaerobic infections of the oral cavity.

Tilmicosin is indicated for the treatment of pneumonia associated with *Pasteurella* spps. and *Mycoplasma* spp. in young cattle.

Lincomycin is effective against Gram-positive bacteria, anaerobes, and *Mycoplasma* but has little activity against Gram-negative organisms.

Clindamycin has more potent antibacterial activity than lincomycin. It is a specially indicated in Staphylococcal osteomyelitis. Lincosamides may cause a fatal enterocolitis in horses. Contamination of feed with trace amounts of lincomycin may cause a drop in milk production, inappetence, diarrhoea or ketosis in cattle.

24.8 Sulphonamides

Sulphonamides form a wide series of antimicrobials that differ in their physiochemical characteristics, hence pharmacokinetics and mode of administration. They act by competing with a tissue factor, p-aminobenzoic acid and therefore inactive in the presence of necrotic tissue. They have a broad spectrum of activity, covering *Chlamydia, Toxoplasma* and *Leptospira spp.* but *Pseudomonas* spp. are resistant. Acquired resistance is widespread. Resistant *Pasteurella* isolates have been reported (Abeynayake *et al.*, 1992).

They are often given IV since the sodium salts are alkaline hence are irritant when administered by IM injection. They are well absorbed following oral administration and diffuse well into body tissues and are partly inactivated in the liver, mainly by acetylation.

24.9 Potentiated Sulphonamides

Sulphonamides may be combined with the folate reductase inhibitor, trimethoprim. Potentiated sulphonamides block sequential stages in the synthesis of tetrahydrofolate and thus have a synergistic action. Independent action of sulphonamides or trimethoprim is bacteriostatic but the combination may be bactericidal. The combination has an extended spectrum being effective on a high proportion of anaerobic bacteria, *Nocardia, Chlamydia* and *Toxoplasma* spp. Plasmid-mediated resistance to trimethoprim is possible. Trimethoprim diffuses well in tissues. The combinations contain the sulphonamide is to trimethaprim in a of ratio 5:1.

Combinations available:

(a) Co-trimazine that contains sulphadiazine trimethoprim (for all spp.)
Dose: cattle -15-24mg/kg. daily IM or IV
WP: 14 days for meat animals, 3 days for milking animals

(b) Combination of sulphadoxine and trimethoprim (for all spp.)
Dose: cattle - 15 mg/kg daily or on alternate days SC, IM or IV.
WP: meat animals 10 days and milking animals 2 days.

(c) Combination of sulphatoxazole and trimethoprim
Dose: cattle - 15mg/kg daily IM or IV.
calves - 20mg/kg daily, oral.
WP: 28 days in meat anmals, not to be used during milking.

24.10 Quinolones

The quinolones are a series of synthetic anti-bacterial agents. The early members of the group (first generation) included nalidixic acid, flumequine and oxolinic acid having activity against Gram - negative organisms, and their use was limited to the treatment of urinary and enteric infections. Further investigations and synthesis has given rise to the second generation quinolones referred to as fluroquinolones.

Fluroquinolone derivatives, such as enrofloxacin and marbofloxacin have a broad spectrum of activity and are well distributed in the tissues. The bactericidal activity is by inhibiting microbial DNA-gyrase enzyme which is responsible for supercoiling of DNA-strands. The antibacterial spectrum covers Gram-positive, Gram-negative bacteria and *Mycoplasma* spps. They are not effective against anaerobes. Fluoroquinolones may inhibit the growth of load bearing articular cartilage and therefore contra-indicated in growing animals.

Enrofloxacin
Dose: cattle - 2.5 mg/kg daily SC.
calves - 2.5 mg/kg. daily by mouth
WP: meat animals, 14 days.

Selected references

Abeynayake, P., Wijewardena, T.G. and Thalagoda, S.A. (1993) In : *Antimicrobial Susceptibility of Pasteurella multocida Isolates.* ACIAR Proceedings No. 43. pp.193-196.

Bishop, Y. (1998) *The Veterinary Formulary* (4th Edition), British Veterinary Association.

Prescott J.F. and Baggot, J.D. (1993) *Antimicrobial Therapy in Veterinary Medicine.* (2nd Edition) Iowa State University Press.

CHAPTER 25

Treatment and Control of Parasitic Infections

Preeni Abeynayake

Introduction

High humidity and temperature in tropical countries provide a favourable environment for maturation of infective eggs and makes them available to infest grazing animals. Antiparasitic drugs are effective for limited durations while maintaining therapeutic concentrations at the site of infestation. This means that it is possible for grazing animals to be re-infested within few days of treatment. Due to this reason, effective use of anthelmintics should be closely related to farm management systems.

25.1 Anthelmintics

The three major groups of endoparasites are nematodes, cestodes and trematodes. The diversity of biological characteristics of the three groups necessitates the use of drugs with different modes of action and distribution patterns. The choice of the drug becomes complicated by the fact that the various developmental stages are lodged in different organs and may not be equally susceptible. Moreover, regular use of anthelmintics has lead to the emergence of drug-resistant parasites. Acquired resistance is now widespread due to animal movement, particularly in sheep and goats. Acquired resistance can be minimised by using the most selective drug at a critical time of a life-cycle thereby minimising the frequency of administration. Yearly rotation of anthelmintics between different classes having different modes of action is beneficial.

For the young stock, strategic deworming programmes involve drenching animals 2 - 3 weeks before the onset of rains. The second dose should be given after allowing one cycle of development to be completed. Well planned drenching programmes based on the epidemiology of the parasite allows the drug concentration to be maintained at a critical time of a parasite's life-cycle, thereby minimising the drenching frequency.

Drenching is the commonest method of treatment but alternative methods that provide persistent drug levels may reduce the work load of the farmer. Modified release intra-ruminal devices (continuous or pulse-release devices) provides medication over 90-135 days, "pour-on" formulations and medicated feed blocks are the other alternatives.

25.1.1 Avermectins

The available chemicals in this group are ivermectin, *abamectin, doramectin* and *epirnomectin.* These drugs have been shown to cause paralysis of parasaties by potentiating the inhibitory neurotransmitter γ-aminobutyric acid (GABA). Avermectins are not effective on flatworms or tapeworms since these helminths do not use GABA as a neurotransmitter. Mammalian GABA-ergic neurones occur in the central nervous system, and the therapeutic amounts of the administered drug is unable to cross the mammalian blood-brain barrier. Hence it is of low toxicity in host spp.

Avermectins are effective against adult and larvae of all major nematodes, including inhibited larvae of *Haemonchus* and tissue dwelling larvae of horse *(i.e. Strongyles)*. In addition, this group of drugs is effective against ectoparasites such as lice, mites, ticks and fly larvae. Because of this activity on ectoparasites this group of drugs are termed endectocides. Avermectins have persistent therapeutic activity to prevent re-infection, for a period after drug administration. The protective duration depends on the specific chemical, formulation, route of administration and parasitic species.

Ivermectin protects cattle against *Ostertagia* for up to 21 days, *Cooperia* for 7 to 14 days (depending on the formulations) and lung worms for 28 days.

Doramectin protects cattle against *Ostertagia* for up to 28 days. *Cooperia* up to 21 days and lung worms for 28 days.

This property of preventing reinfection after treatment is utilised in strategic programmes for prevention of parasitic gastro-enteritis in calves. However, due to the residual activity, these drugs lead to long withdrawal periods before slaughter and are contraindicated for lactating animals.

25.1.1.1 *Ivermectin*

Indications:

- gastro-intestinal roundworms and lungworms in ruminants.
- nasal bots in sheep

Contraindications:

- administration of ruminant boluses to non-ruminating stages; that is calves less than 12 weeks of age.
- lactating animals.

Dose: Cattle, "Pour-on" application 5mg/kg.
SC injection 2mg/kg
Modified release bolus, 1.72g over 135 days per 100-400kg, BW

WP: 21 days for animal for slaughter
28 days before calving

Preparations:

(a) Ivomec injection (MSD Agvet)
10 mg/ml in 50 ml, 200 ml, 500 ml. multidose vials.

(b) Ivermectin 1% (Dynamic pharmaceuticals)
as 20 ml multidose vials.

(c) Ivotek (Star Laboratories Pvt. Ltd., Pakistan)
1% ivermectin injection.

25.1.1.2 *Doramectin*

Preparations: Dectomax: Pfizer (10 mg/ml solution)
Dose: 1 ml/50 kg, IM or SC
WP: 35 days.

25.1.2 Milbemycins

The pharmacology of milbemycins is similar to that of avermectins. The chemicals moxidectin and milbemycin oxime are available for therapeutic use. Moxidectin injectables contains 10mg/ml (1% solution). Withdrawal period before slaughter is 45 days. It is not to be used in lactating animals within 60 days before calving.

25.1.3 Benzimidazoles

Benzimidazoles such as *fenbendazole, mebendazole, oxfendazole* and *albendazole* have the same mode of action. They disrupt parasite energy metabolism by binding to tubulin, an essential protein constituent of the parasite responsible for uptake of nutrients. Febantel is a prodrug (probenzimidazole) which is converted to fenbendazole within mammalian tissue.

The anthelmintic activity of benzimidazoles is related to the duration of therapeutic blood concentration. However, a single dose is sufficient in ruminants because the rumen acts as a drug reservoir. Most of these chemicals are effective against larval and adult roundworms including lungworms. Triclabendazole is effective against both immature and adult *Paramphistomes* but has no activity on nematodes. Albendazole has been found to be teratogenic if given during early stages of pregnancy.

Benzimidazole resistance has been found in some strains of *Haemonchus* in goats and sheep. Resistance to one benzimidazole confers cross resistance to other drugs in the same chemical group, having same mode of action. The onset of resistance can be delayed by taking precautions to administer the correct dose. The dose should be based on the heaviest animal of the herd. Accuracy of the drenching equipment is important. If a part of the dose is spilt, the full dose has to be readministered. Newly purchased animals should be quarantined, so that they are not allowed to bring in any resistant worms.

25.1.3.1 *Fenbendazole*

Indications:	Gastrointestinal round worms Hypobiotic larvae of gastro intestinal worms Lungworms
Contraindications:	(i) Should not be given within 14 days of treatment for liver fluke infestation, due to the possibility of potentiating of toxic effects of two drugs. (ii) Should not be given when presence of benzimidazole resistant worms are suspected.

Dose : Oral - Cattle - 7.5 mg/kg single dose
- Sheep - 5mg/kg single dose

25.1.3.2 *Febantel*

Indications: - Gastrointestinal round worms
Lungworms
Tapeworms (in sheep)

Dose: - Cattle 7.5 mg/kg orally
- Sheep 5mg/kg orally

Products: Rintal 10% granulate (Bayer)
15g containers

25.1.3.3 *Albendazole*

Indications: Gastrointestinal roundworms
Lungworms
Tapeworms
Adult *Fasciola*

Contraindications: Concurrent administration with other ruminal boluses
During mating period and early gestation.

Warning: Teratogenic

Dose: Cattle - 7.5 mg/kg for roundworms and tapeworms,
10mg/kg for adult flukes
Sheep - 5 mg/kg for roundworms and tapeworms
7.5 mg/kg adult flukes

Preparations:
(i) Analgon: Wockhardt
Analgon oral suspension, containing 25mg/ml in containers of 60 ml, 1 L, 5L.
Analgon tablets, contains 1.5g/tablets in packs of 12 tablets.
(ii) Alzole: Unjha Formulation Ltd.
Alzole bolus 1.5g
Alzole suspension 50 ml bottles.
(iii) Vermiprazole: (Laboatorios Hipra, Spain)
oral suspension of 10% albendazole in 100 ml and 1 L containers.

25.1.3.4 *Triclabendazole*

Indications: Immature adult *Fasciola* in ruminants and horses

Dose: Cattle - 12 mg/kg, orally
Sheep -10mg/kg, orally

Products: (i) Fasinex 10%: (Ciba)
for cattle oral suspension of 100 mg/ml in containers of 1L and 2.5 L.
WP: For slaughter 28 days
Not to be used in milking cows or within 7 days prior to calving.

(ii) Fasinex 5%: (Ciba)
for sheep, oral suspension of 50mg/ml in
containers of 1L, 2.5 L and 5 Ls.
WP: For slaughter 28 days
Not to be used in milking cows or within 7 days prior to calving.

25.1.3.5 *Mebendazole*

Indications: Gastrointestinal roundworms in sheep
Lungworms in sheep
Tapeworms in sheep
Side-effects: Occasional mild diarrhoea
Dose: Sheep - 15 mg/kg, orally
Products: Wormin 500 bolus Vet. (Cardilla Laboratories)
Available as (500 mg boli)

25.1.3.6 *Oxfendazole*

Indications: Gastrointestinal roundworms, lungworms, tapeworms and hypobiotic larvae.
Contraindications: (i) Ruminal boluses should not be given to calves less than 12 weeks age.
(ii) Concurrent administration with other ruminal boluses.
Dose: Cattle: 4.5 mg/kg as a single dose, oral
Sheep: 5mg/kg as a single dose, oral

25.1.4 Imidathiazoles

These chemicals act by interfering with parasite nerve transmission, causing muscle paralysis and rapid expulsion.

Levamisole is the commonly used drug, effective against adult and larvae of gastro-intestinal roundworms and lungworms. The therapeutic index is low in mammals and could cause adverse reactions such as salivation and muscle tremors. Levamisole is an "immunostimulant" able to modulate cell mediated immune response by restoring depressed T-cell function.

Contraindications:
(i) administration within 14 days of treatment with organophosphorus compounds or diethylcarbamazine.
(ii) application of 'pour-on' formulations to pet animals

Side-effects:
(i) transient coughing
(ii) overdose may cause transient muscle tremors
(iii) salivation, nervous symptoms and colic

Warning: operators should wear protective clothing

Dose Cattle - for round worms
7.5 mg/kg oral or SC
10mg/kg by 'pour-on'
Products: (i) Nilverm (Pitman-Moors)
strength of 80g/L oral solution
WP: Cattle -14 days for slaughter, should not be used in lactating cows.
Sheep - 21 days for slaughter
(ii) Levoplix (ERFAR SA Pharmaceutical, Greece) contains levamisole 10% soluble powder 20g sachet.

25.1.5 Tetrahydropyrimidines

This group of anthelmintics interfere with parasitic nerve transmission as cholinergic stimulants, leading to neuromuscular paralysis. Effective against adult and larval gastrointestinal roundworms.

Available chemicals are:

Morantel citrate monohydrate, oral suspension
Dose: Sheep - 29.7mg/ml

Morantel tartrate, slow release bolus
Dose: Cattle - 11.8g delivered for 90 days, for cattle more than 100kg BW
WP: nil
(Morentel is available as a modified-release ruminal bolus).

Pyrantel embonate, oral paste
Dose: Horse - 439mg/g of base material.
Pyrantel may be effective against tapeworms in horses.

25.1.6 Salicylanilides

They are effective against flukes and act by uncoupling oxidative phosphorylation during energy metabolism. Effective on *Haemonchus* and on benzimidazole-resistant nematodes. Strongly plasma bound group of drugs, with the exception of niclosamide, they therefore have a strong effect on blood sucking parasites.

25.1.6.1 *Closantel*

Indications: Immature and mature *Fasciola*
Oestrus ovis and *Haemonchus* in sheep
Dose: Sheep - oral 10 mg/kg.
Preparations: Flukiver (Janssen), oral suspension, 50mg/ml,
pack sizes 1L, 2.5L and 5L
WP: Sheep 42 days before slaughter, not be used in milking animals.

25.1.6.2 *Oxyclozanide:*

It is mainly active against adult flukes. The drug is distributed to liver, kidney and excreted in bile.

Indications: Adult *Fasciola* in ruminants (not effective on immature stages of *Fasciola*) and immature *Paramphistomes*

Side effects: Occasional increased defecation and inappetance in cattle

Dose: Cattle - oral 10mg/kg
Sheep - oral 15mg/kg.

Products: (i) Zanil Fluke Drench (Mallinkrodt Veterinary)
oral suspension containing 34mg/ml in containers of 5L and 10L.

WP: Cattle - 28 days before slaughter
- 3 days for milking animals
Sheep - 28 days before slaughter
- not to be used on milking sheep

(ii) Tolzan F200 Vet (Hoechst Roussel),
tablets containing 200 mg oxyclozanide in
pack size of 100 tabs (10 strips of 10 tabs)

(iii) Tolzan F Vet (Hoechst Roussel)
Oral suspension containing 3.4g/100 ml in 1L packs

(iv) Tolzan F Vet. Hoechst Roussel, bolus containing 1g oxyclozanide,pack size: 30 boli (10 strips of 3 boli)

25.1.7 Anthelmintics in fixed-dose combinations

25.1.7.1 *Nilzan*: Mallinkrodt Veterinary
levamisole 30 mg and oxyclozanide 60 mg.

25.1.7.2 *Systamex Plus*: Pitman Moore
Oxfendazole 22.65 and oxyclozanide 62.50 mg

25.2 Ectoparasiticides

This group of drugs is available for systemic administration and for topical application by various methods including dips, spray, 'pour-on', dusting powder and as ear tag.

Insecticides may be toxic to animals and the operator. Care should be taken during handling of products. Label recommendations should be strictly followed for storage, use and finally disposal of unused material and empty containers.

25.2.1 Avermectins and Milbemycins

25.2.1.1 *Ivermectin*

Indications: Lice in cattle and warble fly larvae in cattle

Contraindications: (i) administration of ruminal boluses to non-ruminating calves.
(ii) calves less than 12 weeks of age
(iii) 'pour-on's should not be applied on wet hair or when rain is expected.
(iv) direct application of 'pour-on' on to mange scab or
(v) areas soiled with mud or dung.
Dose: (i) 'pour-on's 5mg/mL, apply -500 μg/kg from head to tail
(ii) Injectable 10mg/mL, administer 200 micrograms/kg

25.2.1.2 *Doramectin*

Indications: Mites, lice and fly larvae of cattle
Dose: SC 200 μg/kg

25.2.1.3 *Moxidectin*

Indications: Mites, lice and warble fly larvae of cattle
Contraindications: Calves less than 8 weeks of age
Dose: SC 200 μg/kg

25.2.2 Amidines

Amidines act on octopamine receptor sites in ectoparasites and give rise to increased nervous activity.

25.2.2.1 *Amitraz*

Indications: Lice, mites and ticks on cattle.
Keds, lice and ticks on sheep.
Contraindications: on horses concurrent treatment with other insecticides.
Dose: Cattle 0.025% solution as a spray
Preparations: Taktic (Hoechst)
Liquid or dip concentrate available at the strength of 12.5% which has to be diluted before use (amitraz: water=1:500 to get 0.025% solution)
WP: slaughter animals - 1 day
milking animals - 2 days

25.2.3 Synthetic pyrethroids

Natural pyrethrins are extracted from pyrethrum flowers. Pyrethroids act on the Na^+ channels of parasitic neurones, causing initial excitement followed by paralysis. In some preparations pyrethrins are combined with piperonyl butoxide to get the synergistic activity. Piperonyl butoxide inhibits the microsomal system of some arthropods and has been shown to be effective against some mites.

25.2.3.1 *Cypermethrin*

Indications: Flies, lice and blow fly larvae
Contraindications: Lambs less than 1 week of age, used during hot weather.

25.2.3.2 *Deltamethrin*

Indications: Lice and flies on cattle
Lice, flies, blow fly larvae, keds and ticks on sheep
Side effects: Minor signs of discomfort in some cattle up to 48 hours after treatment.
Warning: Some operators may get transient tingling sensations on contact with skin. Operators should wear protective clothing and avoid handling recently treated animals.
Dose: cattle, 'spot-on' application, 10 ml. of 1% solution.

25.2.3.3 *Flumethrin*

Preparations: Bayticol (Bayer)

25.2.3.4 *Permethrin*

Indications: Flies and lice in cattle
Contraindications: Calves less than 1 week of age.

25.2.4 Cyromazine

It is an insect growth regulators. Effective against blow fly larvae on sheep and lambs. The use of a 'pour-on' preparation has the advantage as its efficacy is not dependent on weather, fleece length and wetness of fleece. The persistence of the drug is such that control can be maintained up to 2 months after single application. The drug can be applied before an anticipated fly challenge.

Indications: Blow fly larvae on sheep
Prevention of blow fly strike on sheep.
Dose: Sheep by 'pour-on' 15-50 ml of 6% solution depending on the body weight.

23.2.5 Organophosphorus compounds

These acts by inhibiting cholinesterase enzyme, thereby interfering with neuromuscular transmission in the ectoparasite.

Acute over dosage or overexposure to organophosphorus compounds in animals and humans is characterised by abdominal pain, diarrhoea, salivation, muscular tremors and pupil constriction. Death may be due to respiratory failure. In cases of poisoning, treatment is aimed at inhibiting the muscarinic effects of acetylcholine with atropine.

Chronic exposure of humans may lead to damage to the nervous system and clinical signs including headache, anxiety and irritability.

25.2.5.1 *Diazinon*

Indications: Blow fly larvae, blow fly strike, keds lice and tick on sheep.
Contraindications: With other organophosphates by dipping or orally.

Selected reference

Plumb, D. C. (1995) *Veterinary Drug Handbook* (2nd edition). Iowa University Press.

CHAPTER 26

Prescribing Gut Active Agents, Antihistamines Diuretics and Corticosteroids

Preeni Abeynayake

26.1 Gut active drugs

26.1.1 Antidiarrhoeal drugs

Diarrhoea is a common clinical sign that can be due to numerous causative factors. Dietary imbalance, hypersensitivity, infections, ingestion, toxins, stress or side effects of administered drugs are some of oetiological factors. Treatment should be aimed at eliminating the causative factor and replacing fluid and electrolytes while undertaking dietary control.

26.1.1.1 *Activated Charcoal*: Activated charcoal primarily has absorbent properties. It is one of the most valuable agents for emergency treatment of certain cases of poisoning because of the broad spectrum of adsorptive activity and rapidity of action. It forms a stable complex with many substances and permits their evacuation from the body. Charcoal preparations vary according to the source of the base material, surface area, capacity for drug binding, and affinity and avidity of binding. Charcoal is usually prepared from lignite, wood or peat. Activation forms more pores and enlarges the surface area. Activation time is directly correlated to the molecular size of compounds adsorbed. Since most drugs are of an intermediate molecular weight, charcoal with pore sizes between 10 and 20°A are most appropriate. Absorption of toxicants is by surface binding, and activity is therefore greatest with small particle sizes. Administration with a purgative, such as sorbitol, is a common practice and this facilitates rapid movement of the charcoal-toxin complex.

Activated charcoal is less effective, if time is allowed for toxin to get absorbed into the system. The recommended ratio of charcoal to the toxicant is 10:1. The presence of food generally decreases its efficacy. Powdered activated charcoal is drenched after suspending in water at the dose rate of 20 - 120 mg/kg. An aqueous slurry of activated charcoal at a strength of 2g in 10ml of water can be given at the dose rate of 2-8/kg for ruminants and 1-3/kg for horses.

26.1.2 Anti-foaming agents

Acute ruminal distension can result in compromise of respiratory and cardiovascular function and requires urgent treatment. In ruminants, medication with or without surgical intervention, may be effective depending on the type of bloat.

26.1.2.1 *Treatment of Bloat:* Ruminal tympany or bloat is caused by the accumulation of gas in the rumen as free gas or as a stable foam (i.e. frothy bloat). The methods of prevention and control of bloat includes limited access to pasture, avoiding finely milled feeds and maintaining the animal on a high fibre diet. Anti-foaming

substances can be included in the feed, drinking water or sprayed on the crops. In the majority of cases, free gas bloat is a secondary complication with other diseases such as oesophagitis, oesophageal obstruction, vagal nerve lesions, ruminitis, tetanus and ruminal acidosis. Treatment of free gas bloat includes ruminal intubation or trocharisation to allow the release of gas.

Frothy bloat is dietary in origin and occurs when leguminous plants or high grain diets, which contain foam-forming agents are ingested. In frothy bloat, a stable foam is produced which traps the gases of fermentation. Small gas bubbles are unable to coalesce thereby preventing removal by eructation. The production of insufficient saliva, which is alkaline, may worsen frothy bloat. Medication of frothy bloat requires the administration of an antifoaming agent to break down the stable foam.

(a) *Oils*: Sunflower oil, arachis oil (peanut oil), can be given through a stomach tube at a dose of 250 ml for cattle, 50 ml for sheep.

(b) *Linseed oil in combination with turpentine oil*: Indicated in frothy bloat due to grain overload.

Warning: Turpentine oil is readily absorbed from GI tract and may taint meat and milk. Absorption may lead to clinical signs of renal and gastrointestinal toxicity including colic, diarrhoea, incoordination and excitement, followed by coma.

(c) *Poloxalene*: This is a non-ionic surfactant used for the treatment and prevention of frothy bloat.

(d) *Dimethiocane*: This is a silicone that can reduce the surface tension of gas bubbles causing them to coalesce.

(e) *Sodium bicarbonate*: Sodium bicarbonate 150 to 200g dissolved in one litre of water, given through a stomach tube will produce a to an alkaline condition in the ruminal contents. Care should be taken, because this treatment may cause further gas production.

26.1.3 Laxatives

Laxatives loosen the bowel contents and induce defecation. Drugs that have a stimulant effect on the intestines are known as purgatives or cathartics.

26.1.3.1 *Lubricant laxatives*: These can soften and lubricate the faecal mass which facilitates its smooth expulsion. Lubricant laxatives line the mucosal surface and may impair absorption of fat-soluble vitamins, and other nutrients or drugs.

For example, the absorption of small amounts of liquid paraffin may lead to the appearance of granulomatous lesions in the intestinal wall and the liver. Paraffins are not effective for treatment of chronic constipation or severe impactions.

Dose: Cattle, 3-4 L/450Kg BW by mouth

Warning: When large amounts are given liquid paraffin should be mixed with ginger or mustard in order to prevent inhalation, except when given by stomach tube.

26.1.3.2 *Bulk forming laxatives*: These compounds take up water in the gastrointestinal tract, thereby increasing the volume of the faeces which promotes peristalsis. It is useful in the management of chronic constipation and when excessive, rectal straining needs to be avoided. Due to their ability to absorb water from the intestinal contents, they are also useful in controlling diarrhoea. Adequate fluid should be given when using bulk laxatives to avoid dehydration and consequent worsening of constipation which may even lead to intestinal obstruction. An example of a bulk laxative is bran which is a water insoluble fibre obtained from the outer layer of cereal grains, usually wheat.

Contraindications:	Abdominal pain, vomiting, intestinal obstruction.
Side effects:	Flatulence, abdominal distension
Warning:	Water should be available at all times.

26.1.4 Modulators of intestinal motility

Atropine sulphate: This is an example of an anticholinergic drug (anti-muscarinic activity) having anti-spasmodic action.

Indications:	An adjunct to gastro-intestinal disorders characterised by smooth muscle spasm. (i) pre-anaesthetic medication (ii) antidote for organophosphorus poisoning.
Contraindications:	Congestive heart failure and intestinal hypomotility
Side effects:	Dry mouth, dilation of pupils, photophobia, constipation, urinary retention and tachycardia.
Dose:	Cattle - 30-60 mg/kg, SC.

26.1.5 Antacids

Antacids are useful in prevention and treatment of mild ruminal acidosis. Excessive carbohydrate intake in the form of grain or soluble carbohydrate overload, will lead to production of large quantities of lactic acid in the rumen, instead of the normal volatile fatty acids, thereby leading to ruminal acidosis. Antacids should be given several times a day because infrequent administration results in rebound acid hypersecretion.

26.1.5.1 *Sodium bicarbonate*: It is water soluble and acts rapidly, producing carbon dioxide. The carbon dioxide may either worsen the pre-existing bloat often encountered in severe ruminal acidosis or may be released by eructation. Gas may accumulate if the rumen is atonic and lead to free gas bloat. Bicarbonate may also be absorbed systemically and produce alkalosis.

Dose: Cattle, 60-120g 2 - 3 times daily
Sheep, 40-60g 2 - 3 times daily.

26.1.5.2 *Magnesium containing antacids* : They are less soluble and therefore long-acting if retained in the stomach. Magnesium containing antacids may acts as laxatives.

(a) Magnesium carbonate

Dose: Cattle 16g

(b) Magnesium hydroxide

Dose: Cattle, 400-450g/450Kg BW, 2-3 times daily
Sheep 10-30g, 2-3 times daily.

(c) Magnesium oxide

Dose: Cattle, 1-2mg/kg

(d) Magnesium trisilicate

Dose: Cattle up to 16g.

26.1.5.3 *Aluminium hydroxide*: It is the drug of choice for ruminal acidosis

Side-effects: Constipation
Warning: Reduces absorption of concurrently given drugs.
Preparations: Aluminium hydroxide powder (non-proprietary)
Dose: Cattle, by mouth 15-30g, 2-3 times daily.

26.2 Antihistamines

Histamine is only one of many autacoids (tissue hormones) involved in hypersensitivity reactions. Antihistamine therapy is symptomatic and must therefore be continued until the clinical signs disappears. Systemic therapy should be continued for about three days. The intravenous route carries the risk of cardiac arrest and is best avoided. Topical application may cause sensitisation.

Antihistamines are most valuable for conditions associated with allergic congestion, oedema, pruritis, serum allergy and insect bites. Conditions associated with tissue destruction are also benefited. Such conditions are, acute septic and gangrenous mastitis, septic metritis, retained placenta, myoglobinuria and azoturia.

Two major histamine receptor types (H_1 and H_2) have been recognised. Contraction of smooth muscles in bronchi, intestines and uterus are controlled by H_1 activity. Increased gastric secretion, stimulation of other exocrine secretions, some instances

of smooth-muscle-contraction inhibition, enhancement of cardiac function and a contribution to sustained dilatation of small blood vessels are important changes mediated by H_2 receptors.

26.2.1 Chlorpheniramine maleate

Indications: Premedication for drugs that may induce an anaphylactic reaction allergic skin disorders

Contraindications: (i) urine retention
(ii) glaucoma
(iii) hyperthyroidism

Dose: Cattle, IM, 1ml/50Kg BW
of the product with the strength of 10mg/ml can be repeated 2 or 3 times daily.

26.2.2 Trimeprazine tartrate (ailmemazine tartrate)

Indications: Allergic respiratory diseases.
Side-effects: CNS depression, drowsiness. laminitis
Contraindications: Not to be given IV
Dose: Cattle, IM, 10ml/250Kg BW with the product having strength of 25 mg/ml.
WP: 48 hrs for meat and milk

26.3 Diuretics

Diuretics are mainly used in veterinary medicine to reduce oedema in cases of heart failure, hepatic disease, cerebral oedema, hypoproteinaemia, inflammation and trauma. It acts mostly by promoting sodium excretion thus reducing the volume of extracellular fluid. Some diuretics have vascular effects. Diuretics are usually classified according to their site of action because this affects the likely side-effects. For example, loop diuretics are much more potent than diuretics acting distally because the loop is the site for greater sodium reabsorption.

26.3.1 Frusemide (furosemide)

This is a loop diuretic, potent and has a rapid onset of action but a short duration of activity. It blocks sodium reabsorption in the Loop of Henle.

Since this is a potent diuretic, administration of excessive doses can lead to hypovolaemia and decompensate renal function. However, their potency allows them to remain effective even when urine production is poor, as in renal impairment. These can increase magnesium excretion and may cause severe potassium loss. Hypomagnesaemia potentiates the cardiac effects of hypokalaemia. Loop diuretics may potentiate ototoxic effects of aminoglycoside antibacterials.

Indications: Oedema, acute pulmonary oedema and cerebral oedema

Contraindications: Renal failure with anuria and acute glomerular nephritis
Side effects: Hypokalaemia, may require potassium supplementation.
Drug interactions: Hypokalaemia may potentiate the toxic effects of cardiac glycosides.
Dose: Cattle - Orally 2 - 5 mg/kg IM or IV, 0.5-1.0 mg/kg.

26.4 Corticosteroids

The adrenal cortex produces steroid hormones which influence carbohydrate and electrolyte metabolism. The major hormones are subdivided into two classes: the glucocorticoids, whose actions are principally on carbohydrate but also on protein and fat metabolism. The mechanism of action of glucocorticoids is given in Fig. 26.1.The mineralocorticoids are responsible for regulating electrolyte and hence water balance.

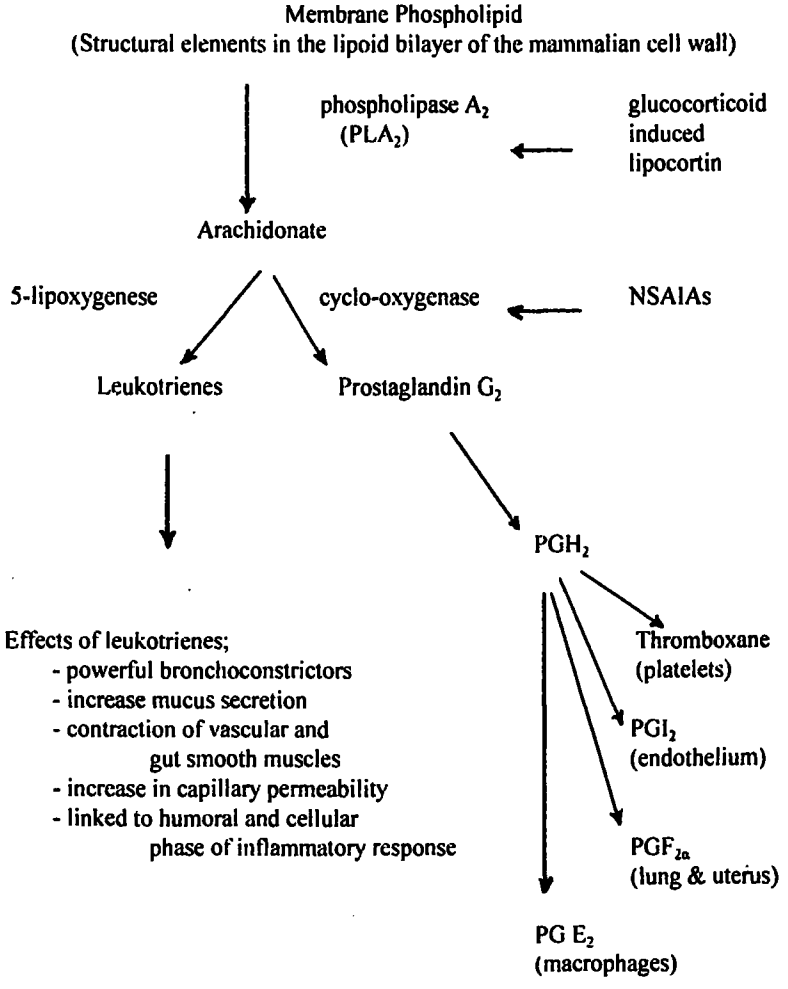

Fig. 26.1 A simplified summary of the arachidonic acid cascade highlighting the site of action of glucocorticoids and NSAIAs.

26.4.1 Pharmacological actions of corticosteroids

The corticosteroid action takes place by enhancing the synthesis of the phospholipase-A_2 inhibitor, lipocortin. The small protein, lipocortin is believed to bind the PLA_2 in the inflammatory cells. By this means, the biosynthetic pathway of inflammatory mediators, archidonates are blocked.

The mechanisms by which the anti-inflammatory actions are exerted on prostaglandin are manifold. According to the Fig. 26.1, leukotriene synthesis is impaired. The long held belief is that the anti-inflammatory action is mediated through vasoconstriction, stabilisation of lysosomal membranes and the reduction of histamine release.

The action of glucocorticoids in suppressing inflammatory reactions may be useful in a wide variety of conditions.

- Respiratory diseases such as chronic pulmonary disease.
- Inflammatory conditicns of the eye, ear and skin
- Treatment of mastitis
- Clinical signs of hypersensitivity disorders including allergic dermatitis and urticaria.
- Early administration of large IV doses may be benefit in acute circulatory failure or shock irrespective of the cause; this may combine with fluid therapy.
- Useful for the induction of parturition during late pregnancy.
- Commonly used in the treatment of ketosis in cattle and goats and also in pregnancy toxaemia in sheep and goats.

26.4.2 Administration of glucocorticoids

Acceptable doses of glucocorticoids vary widely depending upon (i) the potency of the drug, (ii) its formulation, (iii) route of administration, (iv) therapeutic objective and (v) nature and the severity of the condition managed. Corticosteroid therapy is palliative rather than curative. This means that symptoms will reappear, unless the primary cause is resolved.

Table 26.1 The relative anti-inflammatory potencies of glucocorticoids.

Drug	Equivalent anti-inflammatory potency
Hydrocortisone	1
Prednisolone	4
Methylprednisolone	5
Triamcinolone	5
Betamethasone	30
Dexamethasone	30

Water soluble forms are readily absorbed and eliminated within 8 to 24 hours.

Examples are sodium phosphate salt and succinate esters. These can be administered IV and are used when high plasma or tissue concentrations are required rapidly such as in the case of shock or allergy. Despite its rapid elimination, the feed back suppression of ACTH may be prolonged. Water insoluble esters include, acetate, adamantoate, dipropionate, isonicotinate, phenylpropionate, pivalate and trioxa-undecanoate. They are less rapidly absorbed and metabolised. Insoluble esters of dexamethasone are usually immediate-acting and effective for 4 - 14 days.

Depots of long-acting corticosteroids such as insoluble esters of methylprednisolone or triamcinolone may be effective for 3 - 6 weeks. During long treatment schedules, lasting longer than 2 weeks, the dose of glucocorticosteroid (e.g. prednisolone) should be tapered off to the lowest clinically acceptable maintenance level with a gradual transition to administration of twice this maintenance dose on alternate days. If treatment is to be discontinued, the dose should be gradually reduced. The use of injectable combination preparations containing a corticosteroid and antimicrobial is not generally justified.

26.4.3 Side-effects of glucocorticoid

Glucocorticoid therapy is usually accompanied by side effects and unwanted effects.

(i) Prolonged corticosteroid treatment with short acting or depot preparations may have feedback suppression on ACTH and may even lead to adrenal atrophy. Unnecessarily prolonged therapy should be avoided in order to minimise precipitating signs of adrenal insufficiency.
(ii) If used on pregnant animals, it could result in abortion.
(iii) When it is used to induce parturition, the therapy is usually associated with an increased incidence of retained placenta in cattle, which may have effects on subsequent fertility.
(iv) It may induce a temporary fall in the milk yield when given to lactating animals.
(v) Catabolic effects of glucocorticoids include muscle wasting, cutaneous atrophy, telogen arrest of hair follicles and delayed wound healing.
(vi) In cases of corneal ulceration, repair of corneal stroma and epithelium is suppressed.
(vii) Contra-indicated in laminitis in horses.
(viii) Chronic use of exogenous glucocorticoids may lead to iatrogenic hyperadrenocorticism.
(ix) Administration of corticosteroid may result in hepatomegaly with concurrent rise in serum-hepatic enzyme concentrations.
(x) Diabetes mellitus may be worsened.
(xi) Gastric and colonic ulceration, sometimes with perforation, may occur if given in conjunction with certain non-steroid anti-inflammatory analgesics (NSAIAs).
(xii) Immunosuppressive effects and modification of inflammatory reactions may facilitate the progression of concurrent infectious disease.
(xiii) Should not be administered in conjunction with a vaccine and it is contra-indicated in laminitis in horses.

26.4.4 Products available

26.4.4.1 *Betamethasone*

Dose and indications:

Cattle - For inflammatory disorders, shock, and ketosis, IM or IV 40-80 μg/kg. For induction of parturition, IM 20-30 mg and repeat after 3 days if required.

Sheep, goats and pigs - For inflammatory disorders and shock IM or IV 40-80 μg/kg.

26.4.4.2 *Dexamethasone*

Note: Dexamethasone 1 mg = dexamethasone acetate 1.1 mg
= Dexamethasone isonicotinate 1.3 mg.
= Dexamethasone sodium phosphate 1.3 mg.
= Dexamethasone trioxa - undeconate 1.4 mg.

Dose and Indications: Injectable form is available at the strength of dexamethasone 2 mg/ml.

Cattle - For inflammatory disorders and ketosis IM or IV, 5-20 mg, daily
For induction of parturition 20-30mg as a single dose.

Sheep - For induction of parturition - IM or IV, 8 - 16mg as a single dose.

Selected References

Brander, G.C., Pugh, D.M., Bymater,R.J. Jenkins, W.L. (1993) *Veterinary Applied Pharmacology and Therapeutrcs.* (5th edition). ELBS, Baillairc Tindall.

Neal, M.J. (1995) *Medical Pharmacology at a Glance.* (2nd edition). Blackwell Science.

Plumb D.C. (1995) *Veterinary Drug Handbook* (2nd edition). Iowa University Press.

CHAPTER 27

Drugs Acting on the Nervous System

D. D. N. de Silva

Introduction

The commonly used drugs which act on the nervous system in ruminants could be categorized as follows:

1. Sedatives/Tranquillizers
2. Analgesics
3. Neuroleptanalgesics
4. Antidotes of sedatives and neuroleptanalgesics
5. General anaesthetics
6. Local anaesthetics
7. Drugs used for euthanasia

27.1 Sedatives/Tranquillizers

Sedatives cause calmness, drowsiness and indifference in an animal. The difference between a sedative and a tranquillizer is not distinct but, in general tranquillizers produce calmness without drowsiness or with very little drowsiness. The degree of sedation or tranquillization depends on various factors such as the type of drug, dosage, route of administration, species, breed, sex, physiological status of the animal, concurrent medication , the amount of stress and the initial excitement of the animal. As a practice, sedatives or tranquillizers should be administered to animals without exciting them. If the animal becomes excited, the dosage requirement is high or the drug may not have the predictable calming effect. Similarly, if the animal is in pain, the sedative/ tranquillizer will not have the desired effect.

General indications for use

(a) Preanaesthetic medication prior to general anaesthesia
(b) Prior to various examination purposes and radiological procedures
(c) Prior to major or minor surgical procedures performed under block or infiltration anaesthsia
(d) To facilitate handling and transportation of animals.

Types of drugs available for sedation and tranquilization

1. Phenothiazine derivatives
2. Alpha 2 agonists
3. Benzodiazepines
4. Butyrophenines

Among these, phenothiazine derivatives and alpha 2 agonists are the most useful agents for use in ruminants.

27.1.1 Phenothiazine derivatives

27.1.1.1 *General effects*

(a) Causes depression of the brain stem which inhibits alertness resulting in sedation.
(b) Potentiation of analgesics and general anaesthetics
(c) Negative inortropic action on the heart.
(d) Moderate antiacetylcholine, antihistamine and anticholine-esterase effects
(e) Alpha adrenergic blockade causing hypotension
(f) Hypothermia

Usually increasing the dose of these agents will not increase the amount of sedation that
results. The phenothiazines are detoxified in the liver and eliminated mainly through urine.

Contraindications:

(i) Do not use in cases of organophosphate poisoning or after recent treatment with organo-phosphate ecto-or endo-parasiticides.
(ii) Do not use or use with extreme caution in cases of hypovolaemic or endotoxic shock.
(iii) Do not use epinephrine to counteract hypotension caused by phenothiazine type sedatives because it will potentiate hypotension. The alpha stimulation (vasoconstriction) provided by epinephrine will be partially obviated by the sedative allowing the beta effect (vasodilatation) to prevail.
(iv) Should not be used in animals with phenothiazine sensitivity.
(v) Intracarotid (intra-arterial) injections must be avoided as it will precipitate convulsions and can cause death.

Phenothiazine derivatives that are available are chlorpromazine HCl, promazine HCl, acepromazine maleate and propyonyl promazine. Among these, acetyl promazine maleate and promazine HCl are the commonly used phenothiazine derivatives. Propionyl promazine is also a useful agent although it is not currently available in Sri Lanka.

(a) Promazine hydrochloride

Frequently used in large animals. Less hypotensive and has fewer side effects than chlorpromazine. Duration of action is about 2 hours after intramuscular injection.

Dose: 0.44-1.1 mg/ kg, I/M (half the I/M dose could be given I/V, but it must be administered as slow injection).

Presentation: "Sparine" (M&B) 25 mg / ml, ampoules of 2 ml.

(b) Propionyl promazine

Indications: (other than general)

(i) Sedation of excited, unmanageable or aggressive animals including zoo animals at animal shows.
(ii) To avoid loss of weight during transport.
(iii) As a sedative before removing foreign bodies in the pharynx.
(iv) To obtain adequate protrusion of penis in standing bulls for examination.

Dose: Cattle - 1.0 - 2.0 ml/100 kg, I/V or I/M of 1% solution.
Sheep/goat - up to 1.0 ml per animal, I/M or S/C of 1% solution

Cattle in standing position, especially in abdominal operations, should receive the lowest dose indicated intramuscularly. Higher doses should be given to extremely nervous animals or if needed to be recumbent.

Presentation: Combelen (Bayer) - 1% solution in 5 ml ampoules and 25 ml vials.

(c) Acetyl promazine maleate

Most potent of the phenothiazine derivatives.

Contradictions: Do not use on pregnant animals.

Dosage: 0.25 - 0.5 ml / 50 kg, I/M or slow I/V.

Presentation: Acepril (Ilium) - 1% solution in 10 and 20 ml vials.

27.1.2 Alpha 2 agonists

Another useful category of agents for sedation of all ruminants and a variety of other species.

(a) Xylazine Hydrochloride

General effects:

(i) Central nervous system depression inhibits impulse transmission to produce muscle relaxation.
(ii) Bradycardia and transient cardiac dysrythmias (usually partial atrio-ventricular block) occur when xylazine is given intravenously.
(iii) In cattle, xylazine produces hyperglycaemia, decrease in plasma insulin and haematocrit.
(iv) The sedative effects are seen in about 10-15 minutes after intramuscular injection and 3-5 minutes after intravenous injection.

Contradications: Do not use on cattle and buffaloes in last trimester of pregnancy.

Dosage: Dose rate varies with degree of the sedation required as shown below.

Degree of sedation required	mg/ 50 kg	ml/ 50 kg
1. Light sedation	2.5	0.12
2. Moderate sedation	5.0	0.25
3. Deep sedation	10.0	0.50
4. Very deep sedation	15.0	0.75

1. Sedation with slight decrease of muscle tone. The ability to stand is maintained.
2. Sedation, marked decrease in muscle tone and some analgesia. Animal usually remains standing but may lie down.
3. Deep sedation, further decrease in muscle tone and useful degree of analgesia. The animal becomes recumbent.
4. Very deep sedation, a profound decrease in muscle tone and a useful degree of analgesia. Animal becomes recumbent.

Presentations: Rompun (Bayer) - in 2% solution of 30 ml vials
- 500 mg powder/pack
Xyly (Ilium) - in 2% solution of 10 ml vials

(b) Detomidine Hydrochloride

Indications:

i. Preanaesthetic medication.
ii. Sedation prior to minor and major surgical procedures.
iii. Sedation for handling and restraint for examinations and transport.

Note: *Detomidine does not appear to have any drastic effect on females in late pregnancy but extensive work has yet to be carried out to prove this.*

Dosage: For cattle and buffaloes - 0.2ml/100kg for mild sedation,
0.4ml/100kg moderate sedation
0.6 ml / 100 kg for deep sedation
0.8 ml/100 kg for very deep sedation

Animals become recumbent with higher dose rates.

Presentation: "Domesedan" - FAMOS - vials of 10 and 20 ml - 1% solution.

27.2 Analgesics

In ruminants, analgesia can be achieved by the use of following groups of agents.

(i) Local analgesics.
(ii) Opioid (narcotic) analgesics.
(iii) Alpha - 2 agonists
(iv) Non - sterordal anti-inflammatory drugs.

27.2.1 Local analgesics (will be discussed under a separate title)

27.2.2 Opioid analgesics

Opioids in the form of opium have been used as pain killers in man as far back as at least 2000 years ago. The refined and processed extracts such as morphine and heroin still have a major role as analgesics. At present, a wide range of both naturally occurring and synthetic opioids that differ in potency, pharmacokinetics and in some cases, side effects are in use. In man, these drugs cause euphoria and addiction resulting from abuse, therefore in order to limit this abuse, these products are treated as controlled drugs.

Indications: (i) For induction of analgesia
(ii) For induction of sedation

Mode of action: They combine with opiate receptors in the brain and spinal cord.

Excretion: (i) Biotransformed by the liver and excreted in the urine.
(ii) Small amounts are excreted unchanged in the urine.
(iii) Portions of some agents are excreted in the faeces.
(iv) The pathways vary with the drug and species.

27.2.2.1 *Morphine*

Indications: (i) For induction of analgesia - pre- or post-operative.
(ii) For induction of sedation

General effects: (i) Suppresses the cerebral cortex producing hypnosis and narcosis.
(ii) Decreases intestine motility results in constipation
(iii) May cause muscle tremors, sweating and excitement.
(iv) Retention of urine.
(v) Respiratory depression.

Dosage: Cattle/sheep/goat - 0.1 - 0.3 mg/kg, I/M.
Analgesia lasts for about 4 hours.

Note: *Morphine has been used in combination with xylazine (Rompun) to mask the undesirable excitatory side effects in horses.*

27.2.2.2 *Pethidine*

This has one tenth the potency of morphine. Indications are as for morphine.

Dosage: Cattle/buffalo - 1 mg/kg, I/M.
Analgesia lasts for 1½ - 2 hrs.

27.2.3 Alpha 2 agonists (see para 27.1.2a)

27.2.4 Non-steroidal anti-inflammatory drugs

The use of this category of drugs are limited in ruminants except for diclofenac sodium as an antiinflammatory drug (predominantly peripheral action).

27.3 Neuroleptanalgesics

This group of drugs consists of combinations of opioids with sedative drugs. When these 2 agents are combined, synergism seems to occur, sedation and analgesia being greater than that achieved by either drug alone. The use of the sedative prevents any undesirable effects that may otherwise occur with opioids alone. The range of use of sedative/opioid mixtures for sedation and control of animals has become very wide. Alpha adrenoreceptor agonists, neuroleptic agents and benzodiazepines have been combined with different types of opioid agonists for the use in animal practice. The most commonly used preparation is the combination of etorphine hydrochloride (morphine derivate) and acepromazine maleate (sedative - phenothiazine derivate) which is marketed as Immobilon.

Immobilon LA: Etrophine HCL 2.45 mg/ml
Acepromazine maleate 10 mg/ml

Indications: Capture and immobilization of wild cattle and buffaloes.

Dosage: Cattle/buffaloes - 0.5 ml/50kg, I/V or I/M
Sheep - 0.25ml/50 kg, I/M

Unwanted effects: Severe respiratory depression.

27.4 Antidotes of sedatives and neurolepticanalgesics

Antogonists of neuroleptanalgesics (e.g. diprenorphine HCl) replaces the drug from opioid receptors and neutralises the action of the opioid fraction in the combination. Antidotes of alpha 2 agonists (e.g. yohimbine HCl) also have a competitive affect on their receptors.

Neuroleptanalgesic/Alpha 2 agonists	Antidote
(i) Etorphine HCl and Acepromazine maleate (Immobilon)	Diprenorphine HCl (Revivon)
(ii) Carfentanyl HCl "Wildnil"	Diprenorphine HCl (Antagonil)
(iii) Xylazine HCl (Rompun)	Yohimbine HCl (Riverzine)
(iv) Detomidine HCl (Domosedan)	Yohimbine HCl (Antisedan)

27.5 General anaesthetics

The use of general anaesthetic is very rare in ruminants mainly because most surgical procedures could be carried out with local anaesthetic methods. In addition, the nonavailability of facilities necessary for maintainance of general anaesthesia and the complications associated with general anaesthesia in ruminants exclude the use of general anaesthetic agents in routine practice. However the following drugs alone or in combination with other drugs could be used to induce general anaesthesia.

27.5.1 Thiopentone sodium

Indications: (i) Induction of general anaesthesia for long surgical procedures.
(ii) As a sole anesthetic agent following premedication with a suitable sedative for short surgical procedures.

Dosage: Cattle (i) 11 mg/kg as a bolus I/V injection if premedicated with a phenothiazine derivative.
(ii) 5 – 6 mg/kg, I/V if premedicated with xylazine HCl

The animal sinks quietly to the ground within 20 -30 seconds and there is a brief period of apnoea. Surgical anaesthesia of about 3 - 4 minutes duration is followed by recovery which is usually complete within 45 minutes.

27.5.2 Ketamine hydrochloride

Ketamine is often used in combination with xylazine in general anaesthesia. Induction and recovery is smooth and uncomplicated when this combination is used.

Dosage: Cattle - 2 mg/kg I/V with prior xylzine at a rate of 0.2 mg/kg I/V.
Calves (1 week - 1 year) - 0.2 mg / kg I / V or 10 mg / kg I / M

Anaesthesia lasts for about 35 minutes followed by complete recovery in a further 80 - 90 minutes.

Sheep - 4 mg/kg with 0.05 mg/kg xylazine HCL I/V or with 2 mg/kg diazepam.

Goats - 4 mg/kg with 2 mg/kg diazepam, I/V or with 11 mg/kg ketamine and 0.22 mg/kg xylazene, I/M

It must be noted that xylazine, ketamine and diazepam are all expensive agents and their use in ruminants may be difficult to justify when inexpensive agents are available.

27.5.3 Chloral hydrate

Indications: Induction of general anaesthesia

Dosage: Cattle - 20 -30 ml / 45.5 kg as 7% solution

A long recovery period and the lack of control over the depth of anaesthesia are the main disadvantages.

27.6 Local anaesthetics

The most commonly used method of anaesthesia in ruminants is local anaesthesia. The agents to achieve local anaesthesia (analgesia) could be used in a variety of ways.

27.6.1 Indications

(i) Surface application to induce analgesia over a small surface area.
(ii) Infiltration or block anaesthesia. e.g. inverted "L" block, paravertibral block, retrobulbar block and different specific nerve blocks.
(iii) Epidural block - posterior and anterior.
(iv) Intravenous regional block.

27.6.2 Mode of action of local anaesthetics

(i) It prevents the generation and conduction of the nerve impulse through interference with the permeability of the cell membrane to sodium
(ii) The local anaesthetic agents are presented as salts of weak acids which dissociate to the active agent in neutral or alkaline media. They are not as effective in an acid medium (e.g. an abscess).
(iii) Small nerve fibres are more susceptible to local anaesthetic agents than larger ones.
(iv) Unmyelinated fibres are in general more sensitive than myelinated fibres.
(v) Sensory and motor fibres both react in the same manner.

The duration of action of local anaesthtics depends on the time required for absorption and metabolism of the agent. The length of action may be prolonged by the addition

of vasoconstrictor agents such as epinephrine. Vasoconstriction delays absorption of the drug from a given area and some anaesthetic agents will produce vasoconstriction by themselves. The addition of hyaluronidase may also make the agent more effective because it will break down the tissue barriers and cause a more diffuse distribution of the agent. These agents can produce systemic reactions, especially when given in high doses or repeated doses or when used on small patients. Local hypersensitivity may also be developed on administration of local anaesthetic agents.

27.6.3 Excretion

Depending on the type of local anaesthetic agent, metabolism of the agents occurs either in the liver or in by plasma pseudocholine esterase. A major portion of metabolites are eliminated by kidneys.

27.6.4 Products available - Lignocane HCL 2% (Gesican)
Lidocane HCL 2% (Xylocaine)
Both these are compatible with epinephrine.

27.7 Muscle relaxants

Muscle relaxants have very little use in ruminant anaesthesia. However, following preparation could be used if the need arises.

27.7.1 Guifenesin (formally called glyceryl guaicolate)

Its mode of action is through its action at the internuncial neurons in the spiral cord and brain stem. It is conjugated in the liver and excreted *via* urine.

Indications: (i) For short surgical procedures in combination with local analgesia.
(ii) As an induction agent for inhalation analgesia.

Dose: Cattle - used as a 5 % solution (50.0 gm/litre) with 5 % Dextrose

Note: *2.0 grams of an ultrashort-acting barbiturate may be added to 50 gms of guifenesin to provide more analgesia and quicker induction.*

27.7.2 Succinylcholine chloride

This is a quaternary ammonium compound that exerts its action at the myoneural junction. It binds itself to the acetylcholine receptors causing depolarization of the muscles. Succinylcholine exerts a longer affect than acetylcholine. The muscle loses its ability to respond to the continued stimulation causing relaxation to occur. This is metabolised by plasma pseudocholine esterase.

Indications: (i) In surgical procedures where profound muscle relaxation is needed.

(ii) Open thoracic surgery-where intermittent positive pressure ventilation (IPPV) is used.

Dosage: Cattle and Sheep - 0.02 mg/kg. Ruminants have low circulating levels of pseudocholinesterase causing succinylcholine to have a prolonged effect and an extremely narrow safety range in those species.

27.7.3 Gallamine triethoiodide

Mode of action: Its action is similar to tubocurarine; a non-depolarizing myoneural blocking agent. It prevents acetylcholine from combining with the receptor sites causing paralysis when 80 - 90% of the receptor sites are blocked. This has a slower onset of action than succinylcholine and it produces 10-20 minutes of relaxation.

Excretion: is *via* urine, unchanged.

Indications: Same as for succinylchloride.

Dosage: Cattle and sheep - 0.55 - 1.1.mg/kg, I/V. Additional gallamine may be given at 0.1- 0.5 of the initial dose.

The reversal agent is neostigmine given at a rate of 0.01 - 0.04 mg/kg, I/V in increments after the administration of atropine at the dose rate of 0.02 mg/kg. I/V. Make sure that a source of O_2 and facilities for artificial ventilation is available before administering muscle relaxants.

27.8 Drugs used for euthanasia

27.8.1 General considerations

Before administering any agent to euthanise animals, be sure that permission (preferably written) of the owner is obtained from and also if parenteral agents are used for euthanasia, make sure that the carcass is properly disposed of and not consumed by other animals or people.

The desirable characteristics of the agent used for euthanaesia

(i) Painless
(ii) Humane
(iii) Rapid
(iv) Easily administered
(v) Inexpensive

27.8.1.1 *Barbiturates*: An over dosage of barbiturate solution causes anaesthesia, paralysis of the respiratory centre and cardiovascular collapse. High concentrations of agents are given intravenously as a bolus injection. Prior tranquillization renders

the procedure smoother. The use of these agents are humane, rapid and painless. The disadvantage may be the cost of a large volume of barbiturate required for ruminants.

27.8.1.2 *Magnesium sulphate*: An over dosage of this agent will cause cerebral depression and respiratory paralysis. A large volume of supersaturated solution is needed to be given as a rapid intravenous injection to be effective. Its use is humane, rapid and painless and less costly. But the requirement of a large volume especially for bovines is a disadvantage.

27.8.1.3 *Strychnine hydrochloride*: It could be given as a intramuscular or intravenous injection. It causes severe tetanic spasms and death occurs due to suffocation without cerebral depression. It is relatively inexpensive since only a small volume is required but it is an inhumane method. It should never be administered in the presence of client.

Selected references

Bishop, Y. (1998) *Véterinary Formulary* (4th edition). Royal Pharmaceutical Society of Great Britain and British Veterinary Association.

De Silva, D.D.N., Dangolla, A. and De Silva, L.N.A. (1991) Preliminary studies on sedative and analagesia effects of Detomidine (Domesedan) in Buffaloes. *Sri Lanka Veterinary Journal* **38,** 26.

Glick, R. (1995/96) *Veterinary Pharmaceuticals and Biologicals.* (9th edition). Veterinary Medicine Publishing Company, Kansas.

Hall, L.W. and Clarke, K.W. (1991) *Veterinary Anaesthesia.* (9th edition). W.B. Saunders Company.

IVS Manual (1996) MIMS Australia, Division of Medimedia Australia Pty Ltd.

Pushpakumara, P.G.A. and De Silva, D.D.N. (1993) Effects of α - 2 adrenergic agonist detomidine HCl (Domesedan) on cattle in Sri Lanka. *Sri Lanka Veterinary Journal.* **40,** 35.

Riebold, T.W., Goble, D.O. and Geiser, D.R. (1987) *Large Animal Anaesthesia - Principles and Techniques.* (1st edition). The Iowa State University Press, Ames.

CHAPTER 28

Body Condition Scoring System for Buffaloes and Cattle

H. Abeygunawardena, B.M.A.O. Perera and J.A. de S. Siriwardene

Introduction

Body condition is a reflection of the muscle mass and fat reserves of the animal body. The energy stored in muscle and reserves is used by dairy animals either when the feed is not adequate to meet the nutritional requirements of animals or when the animals cannot consume enough feed to satisfy the special demands for nutrients such as in high producing animals in peak lactation. Generally, animals lose body condition during early lactation and or when feed is inadequate or of poor quality.

In smallholder farms, poor body condition is evident in animals that are underfed and as a result, their productivity and reproductive performance decline below the optimal level. Under local management conditions, this situation arises due to the scarcity of grazing grounds and fluctuation of the quality and quantity of green feed available, due to seasonal variation in rainfall. Traditionally, concentrate feeds such as coconut poonac and rice bran are fed as supplements to overcome the inadequacy of forage, but generally they are given only in limited quantities, due to the high cost of concentrates in comparison to the price received by farmers for milk. The situation is further aggravated by the lack of a proper understanding of the potential value of alternate low-cost feed resources, such as tree fodder and rice straw, to meet the shortfall of nutrients. The procedure to adopt in the formulation of a ration in the most cost-effective manner is given Chapter 6. The methods of utlizing non-conventional feed resources such as rice straw and tree fodder to meet feed shortages is discussed in Chapters 3 and 4. The methods of incorporating strategic feed supplements such as urea and molasses to improve the digestibility and utilisation of low quality feed material are discussed in Chapter 5.

The most important criteria for determining the efficiency of dairy buffalo and cattle management systems and also in diagnosing causes of infertility, is the nutritional status of the animal. The conventional method that is used to assess whether animals receive adequate levels of nutrients is by matching the nutritive value of forages and concentrates offered in terms of TDN and DCP with the TDN and DCP requirements of the animal. This is a very cumbersome and expensive procedure. Such a technique has very little value in field situations. The other method is to compare the body weight of the animal with the expected body weight for that species. But, the body weight is an unsatisfactory indicator of the nutritional status of animals due to obvious reasons. Therefore, in order to meet the need for a simple and convenient method of estimating the nutritional status of animals, scientists have developed a system called the body condition scoring system which could be used easily by veterinarians, extension workers and also by farmers.

28.1 Body condition scoring system

The body condition scoring system is based on a simple numerical scale which brings out a relationship between body muscle mass and fat cover on the one hand and the nutritional status of an animal on the other. This relationship is made use of in this procedure, since it has been shown that the variation in the of body condition score (BCS) influences the efficiency of production, reproduction and also the health of the animal. It is a more precise measurement than body weight for judging the changes in nutritional status and health of the animal, as body weight can be affected by genotype and factors such as rumen fill and the stage of pregnancy.

The BCS is a very valuable tool by which a veterinarian can assess the nutritional status of an animal in many clinical situations. This information is also useful to smallholder farmers who could use the BCS as a guide in feeding and management of buffaloes and cattle for optimum growth, reproduction and production. This will also allow the farmer to obtain the best economic returns from his buffalo and/or cattle herd.

28.2 Assessment of body condition score of animals

The scale ranges from one to five. It is based on the visual observation and palpation of two or more of the following four points on the animal as shown in Plate 1 and 2.

(i) The area on either side of the tail-head and rectum
(ii) The pin bones (Tuber coxae)
(iii) The bones on the spine (Thoracic vertebrae)
(iv) The short ribs (Lumbar transverse processes)

Plate 1

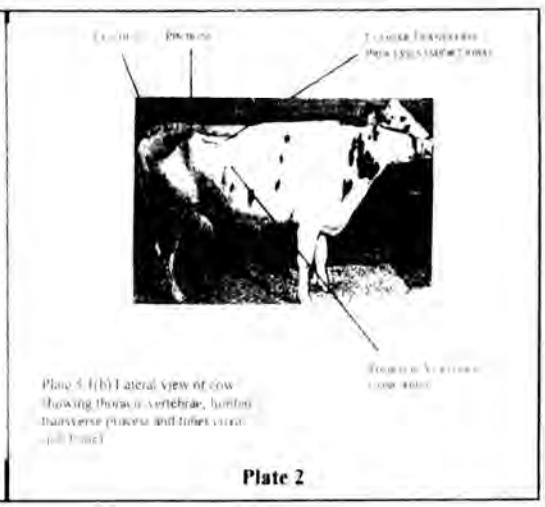

Plate 2

The appearance and the palpable features of these areas reflect the degree of accumulation and mobilisation of body nutritional reserves, mostly as body fat. This is directly related to the nutritional status of the animal, which depends on feed intake and utilisation.

The method of assessing body condition is easy and is based on visual observation and handling of certain sites on the body of the animal like the backbone, loin and rump areas. Generally, the pin bone, hipbone, the top of the backbone and the ends of the short nibs do not have much muscle tissue covering them. The cover that is seen or felt is a combination of skin and fat deposits (Fig.28.1). The assessment of the body condition is made by (1) pressing the finger tips on the backbone, pin bone and hip bone and (2) by gripping the loin of the animal where the short ribs project from the backbone, first in front of the hips, with the fingers on top of the loin and the thumb curved around the ends of the short ribs. The finger tip pressure will give a good indication of the amount of fat cover.

The procedure for determining the condition score is as follows:

(i) Note the appearance of the area on either side of the tail-head and rectum. Are there deep cavities or is the area well filled ?

(ii) Palpate the pin bones. Are the borders sharp or rounded ?

(iii) Note the appearance of the bones on the spine. Are they individually visible or covered by muscle and fat ?

(iv) Palpate the ends of the short ribs as shown in the Fig. 28.1. Are they sharp and covered only by skin, or rounded and covered by fat under the skin?

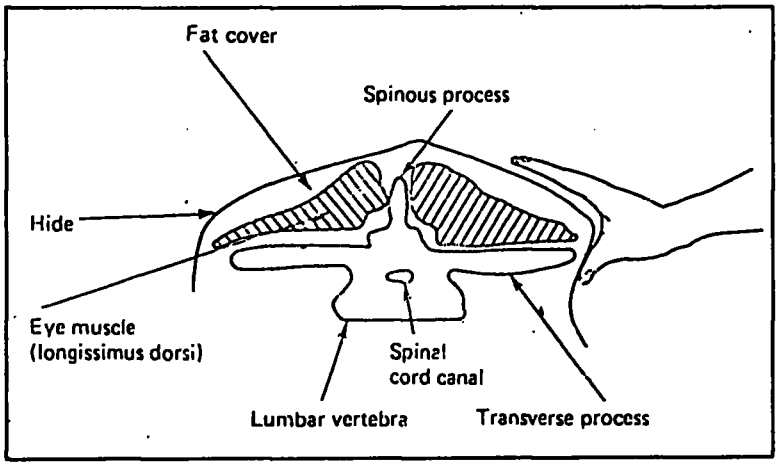

Fig. 28.1 The technique of body condition scoring of cattle over the lumbar vertebrae.

28.3 The body condition scale

28.3.1 Body condition scale for cattle.

Score	Posterior view	Lateral view	Visual and palpable features
1	 **Plate 3a**	 **Plate 3b**	An emaciated animal. Cavities on either side of the tail-head are very pronounced; borders of pin bones are very sharp; individual bones of spine and their borders are visible; ends of short ribs are very pronounced and can be easily palpable; no fat layer under the skin
2	 **Plate 4a**	 **Plate 4b**	An emaciated animal. Cavities on either side of the tail-head are very pronounced; borders of pin bones very sharp; individual bones of spine and their borders are visible; ends of short ribs are very pronounced and can be easily palpated; no fat layer under the skin
3	 **Plate 5a**	 **Plate 5b**	A very lean and healthy animal. Cavities on either side of the tail-head are rounded; individual bones of spine are not visible and muscles are detectable over the bones; ends of short ribs can be still palpated with firm pressure; a medium layer of fat under the skin
4	 **Plate 6a**	 **Plate 6b**	A fat animal. Cavities are filled out; borders of pin bones are rounded; individual bones of spine are not visible and there is muscularity over the bones; ends of short ribs can only be palpated with strong pressure as they are covered with a thick layer of fat under the skin
5	 **Plate 7a**	 **Plate 7b**	An obese animal. Cattle in this condition are usually not found in rural farms. Cavities are not present; borders of pin bones are very rounded; individual bones of spine are not visible and there is abundant muscle and fat over the bones; ends of short ribs are not palpable as they are covered with a very thick large of fat

28.3.2 Body condition scale for buffaloes

Score	Posterior view	Lateral view	Visual and palpable features
1	 **Plate 8a**	 **Plate 8b**	An emaciation animal. Cavities on either side of the tail-head are very pronounced; borders of pin bones very sharp; individual bones of spine and their borders are visible; end of short ribs are very pronounced and can be easily palpated; no fat layer under the skin
2	 **Plate 9a**	 **Plate 9b**	A thin animal. Cavities are less pronounced; borders of the pin bones are sharp; individual bones of spine are less visible; ends of short ribs are less sharp but can be palpated; thin layer of fat under the skin.
3	 **Plate 10a**	 **Plate 10b**	A lean and healthy animal. Cavities are not present; borders of pin bones are rounded; individual bones of spine are not visible and muscles are detectable over the bones; ends of short ribs can still be palpated with firm pressure; a medium layer of fat under the skin.
4	 **Plate 11a**	 **Plate 11b**	A fat animal. Cavities are filled out; borders of pin bones are rounded; individual bones of spine are not visible and there is muscularity over the bones; ends of short ribs can only be palpated with strong pressure as they are covered with a thick layer of fat under the skin.
5	 **Plate 12a**	 **Plate 12b**	An obese animal. Buffaloes in this condition are usually not found in rural farms. Cavities are not present; borders of pin bones are very rounded; individual bones of spine are not visible and there is abundant muscle and fat over the bones; ends of short ribs are not palpable as they are covered with a very thick layer of fat.

28.4 What is the optimum condition score ?

(i) It is recommended that animals should have a condition score of above 3 at calving. This can be achieved by drying off the animal two months before the next expected calving date and providing adequate feed during these two months. This will ensure that sufficient body reserves will be available for milk production after calving.

(ii) After calving, animals usually lose body condition during the first month or two. The quantity of feed provided should be adequate to ensure that the body condition score does not drop below 2, and that it begins to improve 1-2 months after calving. This will ensure efficient and economical milk production and early commencement of sexual cycles.

(iii) The optimum body condition score for dairy cows is between 3 and 4 as shown in the graph below (Fig. 28.2).

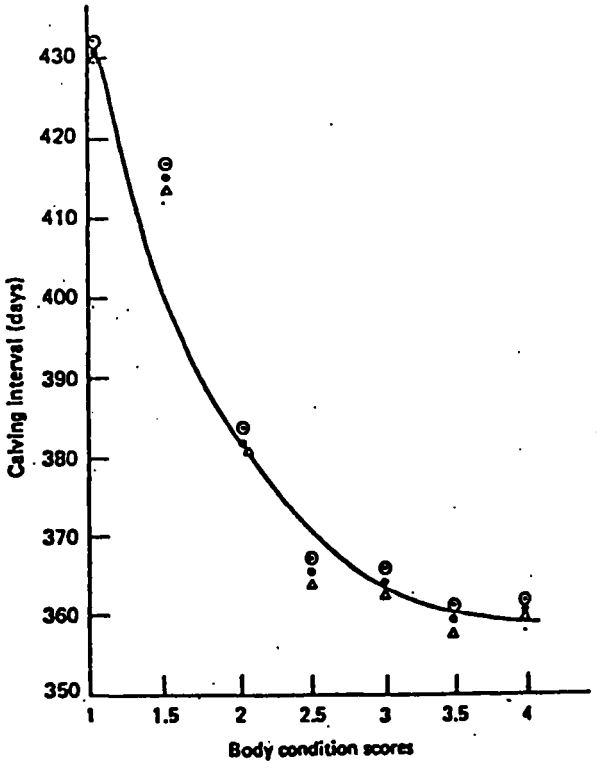

Fig. 28.2 Relationship between body condition score and fertility

As shown in Fig. 28.2 maintaining an animal at a body condition score of 4 or above is not advantageous in terms of production or reproductive performance. It has been

shown that cows will show a diminishing response to an incremental increase of feed (mainly concentrates), until they reach the maximum production potential. But, this level of feeding is not economically advantageous.

The BCS of cows must be determined regularly to assess the changes in body fat resources during each stage of the lactation period. The best procedure is to score the cow at the beginning and at the end of the dry period and at least 4 to 5 times during the lactation period. Such an assessment will give the veterinarian and the farmer an indication of the adequacy or otherwise of the feed supplied and take corrective measures when necessary to restore the body condition back to the recommended score.

Selected reference

Peters, A.R. and Ball, P.J.H. (1995) *Reproduction in Cattle*. (2nd edition) Blackwell Science Ltd. UK.

CHAPTER 29

Management of Buffaloes and Dairy Cattle

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Introduction

The efficiency of a livestock farm operation depends heavily on good management of the stock and other farm resources. To achieve the desired management efficiency, the farmer invariably has to pay adequate attention to the following important aspects: (i) animal handling and management, (ii) feeding and nutrition, (iii) reproductive management (iv) health care and (v) in the case of buffaloes, the regulation of body temperature.

It must also be recognized that the biological and physiological needs of dairy cattle and buffaloes vary according to the breed type, age, sex, reproductive status, production level, status of health and environment. It is therefore imperative that these diverse requirements should be taken into consideration, in the care and management of the different categories of buffaloes and cattle on farms: (1) calves (i.e. neonatal calves and pre-weaner calves), (2) growers (prepubertal heifers, pregnant heifers) and (3) adults (i.e. pregnant cow, parturitient cow and postpartum cow).

In this chapter, an attempt has been made to provide basic guidelines on the more important aspects of dairy cattle and buffalo management, from the new born calf to the adult, reflecting various age groups and physiological states. Repetition of some of the material provided in previous chapters will be evident. This has been done intentionally to reiterate the important aspects of buffaloes and cattle farming and also for the convenience and benefit of the reader.

29.1 Management of calves

29.1.1 Management of the new born calf

At parturition, the neonatal calf for the first time is exposed to a very stressful environment compared to the protective environment inside the mother's uterus. Apart from necessary adjustments in respiration and nutrition, the neonatal calf has to survive in the face of challenges from disease causing agents. The chances of infection are much greater in the neonate, if calving had taken place under unhygienic conditions and where the intake of colostrum was inadequate or delayed. Therefore, it is very important to ensure that calving should take place under clean conditions, free of possible contaminants. Soon after birth, the placental tissue should be removed and the calf cleaned with dry straw, grass or a clean piece of cloth. At this time, mucus from the nostrils should be cleaned to enable the calf to breathe freely. If the dam attempts to lick the calf, this should be encouraged as this will stimulate breathing in the neonate and also the maternal instinct in the cow, in addition to the beneficial effects of cleaning and drying on the calf. It is important to record the birth weight of the calf and subsequently it's body weight at regular intervals to monitor the growth rate. The navel cord must be severed by gentle stretching and if necessary by cutting

it with a sharp clean instrument. A suitable disinfectant like tincture of iodine should be applied on the wound to prevent infection. Application of margosa oil around the umbilicus would be useful as a fly repellant.

The newborn calf will normally begin to assume a standing posture and begin to suckle the mother within 1-2 hours of birth. However, if it does not do so, the calf should be assisted to suckle the dam. It is important to ensure that the baby calf gets the first milk (colostrum) from the dam within the first few hours of life. The calf should be allowed to suckle freely during the next 3-4 days. Colostrum is essential to the new born calf for its nutrition and also to develop resistance to disease, as it is rich in antibodies against common as well as specific pathogenic organisms. In the baby calf, most of the colostrum consumed during the first 36 hours of life is absorbed directly through the intestinal wall. Its absorption is impaired thereafter as the intestine loses the capacity to absorb large molecules. Colostrum also has laxative properties which facilitate the removal of meconium (first faeces) from the gut within a few hours of its ingestion. The daily colostrum requirement is about 5-8 % of the body weight of the calf. Hence the ingestion of colostrum in early life is of vital importance to bring up a healthy calf. The baby calf should also be provided with warm conditions, dry bedding and clean drinking water.

29.1.2 Management of calf - from day 7 to weaning age

29.1.2.1 *Nutrition management*: Milk forms the bulk of the diet in the young calf, and should therefore be provided in adequate amounts to ensure satisfactory growth. In general, a calf would require milk at 10% of its body weight to meet its daily nutrient requirements. This volume of milk may be supplied twice a day.

Suckling is the most convenient and common method of calf feeding, practised by the small holder farmer. However, suckling should be controlled to avoid over-feeding of the calf. It is best to limit milk feeding to twice a day (i.e. once at each milking). Research has revealed that limited suckling also helps to reduce the time interval between calving and first oestrus. In practice this is done in two ways. In one method, three quarters of the udder are milked completely and the fourth quarter is left for the calf (depending on the milk yield of the cow and the requirement of the calf). The quarter left for the calf can be changed rotationally at the subsequent milkings. The other method is to milk all four quarters, leaving a portion (about one fourth) in the quarters for the calf. The calf will be able to extract the milk left over in the udder.

Tender grass and hay (50-100g) may be introduced on or about day 10 to allow for the development of the rumen in the young calf. Concentrate feeds like a calf starter (commercial premixes), coconut poonac or rice polish may be provided to supplement the diet at about two weeks of age, particularly for fast growing animals of *Bos taurine* types or their crosses. The daily allowance of concentrates may be increased gradually to 500-750 g/day at two months of age. The weight of the calf should be recorded at least once a month, to determine its growth rate. Gain in weight of a calf should generally be about 400-500 grams per day. This would depend very much on the genetic composition, the level of feeding and management and the environment.

29.1.2.2 *Weaning:* When the calf is about 10-12 weeks of age it may be weaned, if it has attained twice the birth weight. At weaning the calf can be fed totally on forage, as milk is not an essential component of its diet. The dry matter consumption should then be about 2% its body weight. In addition to forage, the calf requires important minerals like calcium and phosphorus as well as trace minerals which can be provided by means of a good quality commercial mineral mixture. Access to clean drinking water should be ensured throughout the day within the calf pen. Provision of dry bedding should be continued and calves should not be subjected to cold draughts. If calves are reared in groups, they should be prevented from licking one another, as this may lead to the formation of hair balls in the rumen.

29.1.2.3 *Health care:* Most calf deaths occur during the pre-weaning period. Deaths could be prevented or reduced by taking appropriate measures to ensure good hygiene in feeding and management. A common problem encountered, in the early life of the calf, particularly of buffalo calves is roundworm infection (*Ascariasis*). The worm eggs are transmitted from the cow to the young calf through the milk. Infected cows do not show any clinical signs of disease. Parasitic eggs produce larvae that develop in the calf to become adult worms within 3 weeks, after which the calf may show signs of disease characterised by lethargy, diarrhoea, weakness and sometimes death. Therefore, strategic de-worming of calves will prevent the infection. All buffalo calves should be dewormed between 10-16 days of age with an effective drug such as pyrental palmoate (Combantrin - 2 tablets of 125mg each).

Another common parasitic problem which occurs in calves (also in buffalo calves) at a young age is coccidiosis. This occurs generally, at the age of about 3-4 weeks. Clinical signs seen are loss of appetite, diarrhoea with blood stained mucus and a rise in body temperature. Prompt advice and treatment should be provided in all such cases in order to prevent calf losses. Sulphadimidine is an effective drug in the treatment of coccidiosis. Other roundworm and tapeworm infections may occur between 1-3 months of age in calves. Affected animals usually show signs of weakness, swelling under the jaw, diarrhoea and a "pot bellied" appearance. If any of these signs are observed early attention should be given through proper diagnosis and treatment. For more details on health care refer Chapters 18 to 21.

29.2 Management of growers (weaning to puberty)

Providing good nutrition is of primary importance for growing calves in order to maintain the desired growth rate and to ensure the development of resistance to infections. Well nourished stock can withstand disease much better than undernourished animals. Since the weaned calf is now able to depend entirely on roughage feed, good quality hay, pasture and fodder should be provided *ad libitum*. The mineral and vitamin requirements of the growing animal should also be provided in the daily diet and this could be achieved by feeding a commercially available mineral mixture. Good quality grass and fodder supplemented with tree fodder would provide enough nutrients to calves with potential for moderate growth rate (e.g. *Bos indicus* dairy breeds and crosses). However, calves with potential for higher growth rates (e.g. pure *Bos taurine* type or crosses >75% *Bos taurine* blood) may need concentrates such as coconut meal, rice polish or compounded feeds to express full

growth potential. These may be given as a supplementary feed if the cost can be accommodated. However, alternative feed resources such as paddy straw supplemented with UMMM feed supplements, tree fodder may be useful in meeting nutritional deficiencies and also during the dry season, when good quality grass is in short supply. Detailed aspects of feeding are described in Chapter 2, 3, 4, 5 and 6 and examples of some practical rations for growing animals are given in Table 6.3 of Chapter 6 in this volume.

Proper nutrition management promotes steady growth of the young and the achievement of early puberty. Attainment of early puberty increases the reproductive and productive life span of the animal and hence brings enhanced economic returns to the farmer. The growth of the animal should be regularly monitored by recording the body weight every month. In general, the growing animal or heifer is expected to gain about 500g/day. Female cattle and buffalo commence sexual activity (i.e. puberty) as they reach two thirds the mature body weight. Hence a calf with higher birth weight and faster growth rate will subsequently attain puberty earlier than the calf of smaller body weight and slow growth rate. In a well nourished buffalo, the first signs of heat occur at 15 -18 month of age and as they reach about 200-250 kg body weight. Similarly a well- nourished female dairy heifer may reach puberty by 12-14 months of age as they reach 200 - 250 kg body weight. After the initiation of the oestrous cycle in the young heifer, heat signs will occur with normal regularity at an average interval of 21-22 days.

29.2.1 Health Care

If the calf sometimes shows poor growth despite proper nutrition, this could an indication of ill health. The veterinarian should establish the cause of the problem and administer specific treatment. Most of the diseases occurring in this age group could be prevented by routine deworming (Table 29.1) and by vaccination to develop specific immunity against common infectious diseases (Table 29.2). Therefore a routine deworming programme should be adopted from two weeks of age and vaccination programs should be adopted from the age of 4 months.

29.2.2 Breeding of heifers

Adequate attention should be paid to breeding of heifers and cows at the appropriate age and time. If a young heifer has not reached the desired body weight for the specific breed or breed type, mating may be postponed until the desired weight is reached. The recommended body weight to breed a heifer is when she reaches 2/3 the mature body weight of the particular breed type. The best time to breed the heifer is 6-18 hours after the onset of heat. If artificial insemination (AI) services are not available, one could resort to natural service (NS) using a superior stud bull. If the heifer does not come to heat in 21 or 42 days after AI or NS, she should be examined for pregnancy 60-90 days after the service.

Table 29.1 Schedule for routine de-worming in buffaloes and cattle

Species and age group		Timing of treatment	Recommended drug and procedure for worming
Buffalo			
	Calves	Day 10-16 of age	Pyrantel Palmoate: 2 tablets or250 mg per calf. One worming is sufficient.
	Adults	In general, adults do not require worm treatment. However, if an animal shows evidence of weight loss, diarrhoea, reduced milk production, veterinary advice is required.	Recommend appropriate veterinary advice and treatment.
Cattle			
	Calves	1st worming at one month 2nd worming at 3 months	Albendazole; half a tablet or 750 mg per calf *or*
		Other recommendations: Calves managed intensively in the wet zone may be de-wormed once in 2-3 months up to 12 -18 months of age.	Febantel at the rate of 75 mg/kg body weight (about 3 grams per calf)
		In areas where worm infestation is endemic; deworm the young animal at the commencement of Yala (April/May) and Maha (September/October) rains and on each occasion follow the same treatment 3 weeks later.	There are other drugs suitable for worming. provide veterinary advice depending on each situation.
	Adults	For young as well as adults, de-worming is required only if the animal shows signs of worm infestation. Veterinarian should examine dung samples and advice the farmer as to whether worm treatment is required or not.	Recommendations should be made in accordance with the requirements in each situation.

Table 29.2 Vaccination schedule against common infectious diseases of buffaloes and cattle

Disease	Primary vaccination (Age)	Secondary vaccination (Age)	Booster vaccination (Frequnecy)
HS	4 months	7 months	12 months + annually thereafter
FMD	4 months	5 months and 7 months	12 months and bi-annually thereafter
BQ	9 months	9 months	12 months and bi-annually (up to 2 years)

29.3 Management of Adults

29.3.1 Pregnant heifers and cows

Feeding: Pregnant heifers and cows need to be provided good care and nutrition. The pregnant heifer now needs nutrition not only for its growth and maintenance, but also for the growing foetus. Lactating and pregnant cows require nutrients for body maintenance, lactation and for the calf. Therefore animals, in either of the above categories should be placed on a higher plane of nutrition. Nutrition requirements of the pregnant heifer/cow are at its peak during the last two months of gestation, because of the rapid growth of the foetus during this period. On the other hand, she has to build up her own body reserves to improve the milk yield and also sustain milk production throughout the duration of the subsequent lactation. Special care must be taken to provide a quality mineral mixture containing Ca, P, Mg and trace minerals to meet the increased demand for minerals and to help build up mineral reserves which would be eventually used during the lactation. If the pregnant animal is a lactating cow, she should be dried off two months prior to calving.

29.3.2 Management of the parturient cow

Parturition, the act of giving birth to a calf is a very critical time in the life of cow. Certain physiological changes occur during the terminal stage of pregnancy indicating the onset of parturition. The changes include relaxation of pelvic and perineum region, engorgement of the udder and swelling of the vulva with a clear mucous discharge. During the last few hours prior to parturition, the cow will urinate frequently, appear to be restless and seek a quiet place to calve down.

The animal should be provided with a suitable environment and comfortable conditions for calving, which includes a clean place with dry bedding. The animal should also be provided clean drinking water and feed. It is a good practice to watch the process of calving in order to provide assistance if the need arises. In a normal calving, the calf will appear at the vagina with the head placed in between the extended fore legs. If any other disposition is observed, it would indicate a difficult calving (dystocia) and veterinary intervention may become necessary to correct the malpresentation and assist in the delivery of the calf. Sometimes calving may be unusually delayed after the appearance of the calving signs described above. If the delay in over 5-6 hours, veterinary assistance will be required. In the case of a normal calving, procedures described earlier should be adopted regarding the care and management of the neonatal calf and the dam.

29.3.3 Management of the post-partum cow

The aim of dairy cow management is to get one calf every year. As shown in Fig.29.1, the cow must resume oestrous activity within 30 to 45 days following calving and conceive before 90 days after calving. This is not a difficult task to achieve.

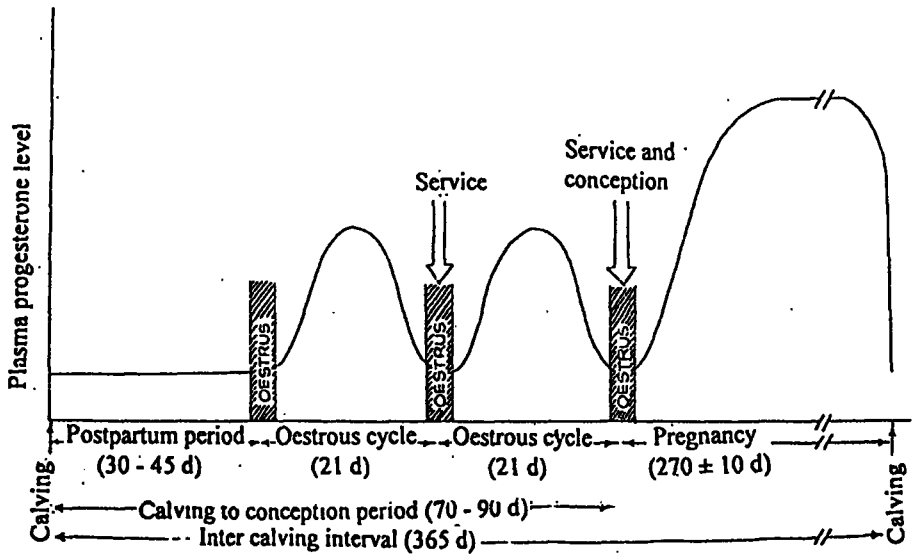

Fig. 29.1 Sexual cycle of a cow depicting postpartum period and gestation

To achieve this goal, several optimum management practices must be followed. Furthermore, it is necessary that possible inhibitory influences must be eliminated or reduced.

(a) As described under "management of pregnant heifers and cows", lactating cows must be dried off at least 8 weeks before the expected date of calving. Similarly in the case of heifers, a "freshing or flushing" period of 8 weeks must be allowed to improve the body condition before parturition. During this time period, a diet rich in fibre, soluble carbohydrates, nitrogenous components and macro and micro minerals must be provided *ad libitum* enable the cow to build its body reserves s to attain a body condition score of 3.5 to 4. Nutrients in feed consumed in excess of the requirements are stored mainly as body fat.

(b) As shown in Fig 29.2, following calving, the body condition of cows decline over the first few weeks, as nutrients are drained from body reserves as milk production increases. As the animal reaches peak production the feed intake even at a maximal level cannot compensate for the increased loss of nutrients from body reserves. During this period, the body reserves (e.g. body fat) are depleted and consequently the cow loses body condition. However, cows with a body condition score of above 3.5 and maintained under good management begin to regain body weight within 30-60 days after calving and resume postpartum ovarian activity, coinciding with the improvement of the body condition (shown at A in Fig. 29.2). Similarly, cows at a body condition score of 2.5 at time of calving will lose body condition after calving, but regain body condition much later than in the previous group. In this example, recovery may take 60 to 90 days after calving (shown at B in Fig. 29.2). Cows that commence lactation or calve down in poor body condition (less than a body condition score of 2), and also are fed poorly during the postpartum period, take much longer to improve the body condition and resume oestrous activity (shown at

C in Fig. 29.2). Perhaps, such animals may never commence ovarian activity and remain in anoestrus as long as the body condition remains below 2. Therefore, cows should be fed with a balanced ration. Guidelines on feeding lactating cows are described in Chapter 6.

(c) Many factors other than poor nutrition have been shown to delay the resumption of postpartum ovarian activity and conception. The major factors that delay the resumption of ovarian activity are old age, suckling by the calf including the cow-calf interaction, stress due to adverse environmental and management conditions and peri-partal diseases, particularly metabolic disorders and uterine infections. The major causes that delay conception are senile and inflamed uterus, poor heat detection and asynchrony between oestrus and insemination and problems related to semen and technician. The causes and appropriate corrective measures that could be adopted are given in the Table 29.3 below.

(d) In the event that a cow does not show oestrous signs by day 60 after calving, veterinary assistance must be provided.

(e) Cows that do not come into oestrus again following natural service by a bull or AI, must be examined for pregnancy within 60-90 days after service. In the event that the cow has not conceived, the farmer should be provide the necessary assistance to help him to avoid economic losses that may arise as a result of conception and pregnancy failures.

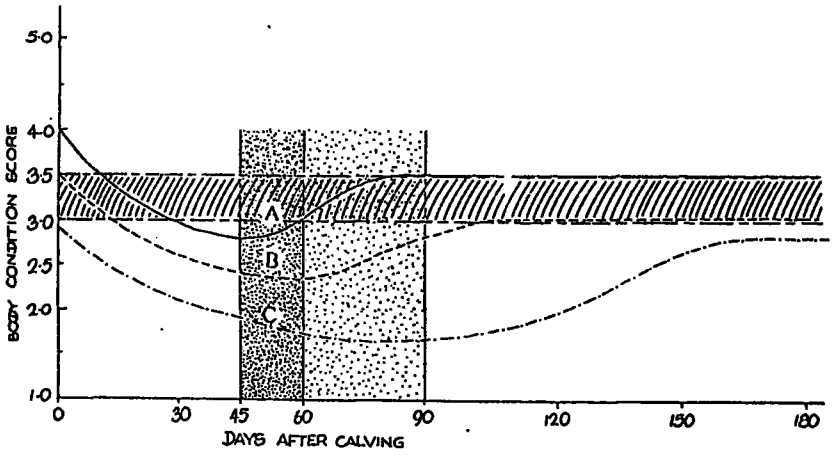

Fig. 29.2 Diagram depicting the possible relationship between body condition score, calving and postpartum period.

Table 29.3 Factors affecting postpartum fertility and remedial measures

Cause	Effect	Remedial measures
1. Old age	Long anoestrous period and poor conception rates	Remove old cows regularly from the herd and replace with young animals.
2. Poor hygiene at calving and peri-parturient complications such as dystocia, retained placenta, metritis, etc.	Long anoestrous period and poor conception rates.	Ensure that calving occurs under hygienic conditions and provide veterinary assistance promptly to correct parturient complications
3. Free suckling by the calf and free interaction between cow and calf	Long postpartum anoestrous period	Adopt limited suckling and allow the calf to suckle the mother only at milking time. In situations where the calf is required for milk let down, introduce the calf to the mother very briefly at the beginning of milking and allow the calf to suckle after milking for 15-30 minutes. The frequency of suckling in this situation is either once or twice, depending on the frequency of milking.
4. Adverse climatic conditions such as hot and humid weather with poor quality feed.	Long anoestrous period	1. Provide shade during the warmer part of the day. Avoid use of corrugated aluminium roofing material, particularly in the low country wet zone, intermediate zone and dry zone. 2. Provide sufficient water throughout the day. 3. In the case of buffaloes provide for wallowing or sprinkling of water at 1-2 hourly intervals, during the warmer part of the day.

Selected references

Abeygunawardena, H., Siriwardene, J.A. de S. and Subasinghe, D.H.A. (1998) *Cattle and Buffalo farming - A Training Manual for Extension Workers*, Colombo, Sri Lanka. National Science Foundation Press. pp 113.

Ibrahim, M.N.M. (1988). *Feeding Tables for Ruminants in Sri Lanka*, Peradeniya, Sri Lanka.

Markham, J.P. and Malcolm, L.R. (1986) (eds.) *The Economics of Tropical Farm Management*. London, Cambridge University Press. pp. 178.

Pasture Research Committee (1982) *A Guide to the Establishment, Maintenance and Utilization of Forage Plants.* Ministry of Rural Industrial Development, Colombo, Sri Lanka.

Subasinghe, D.H.A., Horadagoda, N.U., Abeygunawardena, H and Siriwardene, J.A. de S. (1998) (eds.) *Water Buffalo-Improved Utilisation through New Technologies.* Colombo, Sri Lanka. National Science Foundation Press. 94 pp.

Chapter 30

Crop-Livestock Integration for Maximising Smallholder Farm Income

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Introduction

Crop/livestock integrated farming refers to the complementary use of crop and livestock farming to maximise the returns from a unit area of land. In this system, crops provide crop residues and fodder resources such as rice straw, maize stover and tree fodder which could be used as animal feed. Animals on the other hand provide, in addition to milk and meat, products like draught power and animal waste (dung and urine). The latter is an excellent source of organic fertiliser to enrich the fertility of the soil. Most importantly, livestock provides a regular supplementary income to meet daily cash needs and hence helps the farmer to avoid falling prey to agricultural debt.

Traditionally, livestock is an important component in all smallholder agricultural pursuits in all agro-ecological zones of the country. In rural mixed farm enterprises, most smallholder farmers engage themselves in crop production as the primary agricultural activity but also rear a few cattle and buffalo cows, at negligible or no input cost. In traditional management systems, the animals are fed on fodder resources on communal land - scrub land, reservations, fallow paddy lands, tank beds and canal banks. Animals, in most instances are let loose for grazing during the day time and herded back home in the evening. This traditional extensive system of management of cattle and buffaloes has continued for centuries until the pressure on land increased with more and more land being brought under irrigated agriculture and for human dwellings. Yet, the value of livestock to rural households is quite significant because livestock is still one of the most affordable and a sustainable ways of enhancing rural household incomes.

Constraints faced by the crop-livestock farmers in different regions of the country can be identified as follows:

(a) In most of the dry and dry-intermediate areas of the country, farmers find it increasingly difficult to provide adequate feed to animals due to the reduction in the extent of communal grazing areas and the restrictions imposed on animal movements. Free grazing animals frequently trespass cropping areas and damage crops, irrigation bunds and canals. This usually results in conflicts among farmers, particularly in new settlement areas such as the Mahaweli development zones.

(b) In most areas of the country, the fodder availability depends primarily on the bimodal rainfall pattern. Most areas encounter fodder shortages during the long dry spells and a consequent decline in the quality of roughage.

(c) The existing stock in most of the areas of the country are poor milk

producers, mainly due to poor genetic make up and poor nutrition.

It is conceivable that the plausible solution to overcome these constraints would be,

(a) to reduce the herd size to manageable numbers in keeping with the available resources
(b) to improve the milk production potential of the animals through genetic upgrading and
(c) to introduce better feeding and management practices

Over the last few decades, a vast amount of research conducted in Sri Lanka and elsewhere have addressed these problems and generated a wealth of information on technologies that could be adopted as appropriate to overcome these above problems. Most of this research and development (R&D) effort was directed towards findings ways and means of overcoming feed shortages and nutrient deficiencies.

Many R & D efforts have shown that the feed shortages can be overcome by:

(a) Improving the fodder resource base in homesteads
(b) Better utilisation of locally available pasture fodder species, tree fodder and non-conventional feed resources (e.g. rice straw).
(c) Rational and economic use of concentrate feed such as coconut poonac and rice bran
(d) The use of strategic feed supplements such as urea, molasses and urea-molasses-mineral multinutrient feed supplements.

Therefore, greater intensification of livestock management practices through increased crop-livestock integration with recycling of by- products and by adopting appropriate stock management practices in feeding, breeding and health care appears to be the solution to the present problems and also to ensure the sustainability of the income generating agricultural activities of peasant households.

The aim of this chapter is to provide information for the development and management of crop livestock integrated farms. Specific guidelines are given for,

(a) Establishment and management of the feed base,
(b) Construction of a cattle shed,
(c) Management of animals including milking and record keeping and
(d) Recycling of crop-residues, agro-industrial by-products and animal waste to ensure optimum returns from smallholder crop livestock integrated farm.

30.1 Forage establishment and management

As the profits from smallholder livestock operations are largely dependent on the cost of feed, the use of expensive concentrate feed must be kept to a minimum level. Green forages (grass, fodder and tree fodder) are the cheapest sources of nutrients in the system. The efficient production of green forage within the system is therefore an essential feature in maximisation of production from crop-livestock integrated farms.

Forage production in an integrated system could be easily incorporated into the homestead through the cultivation of fodder, grasses and fodder tree in the following manner as illustrated in Fig. 30.1.

- Fodder trees along the main boundary fence
- Fodder trees as internal hedges / hedgerows
- Fodder grass cultivation in suitable areas around the cattle shed
- Pasture cultivation in identified area for rotational harvesting.

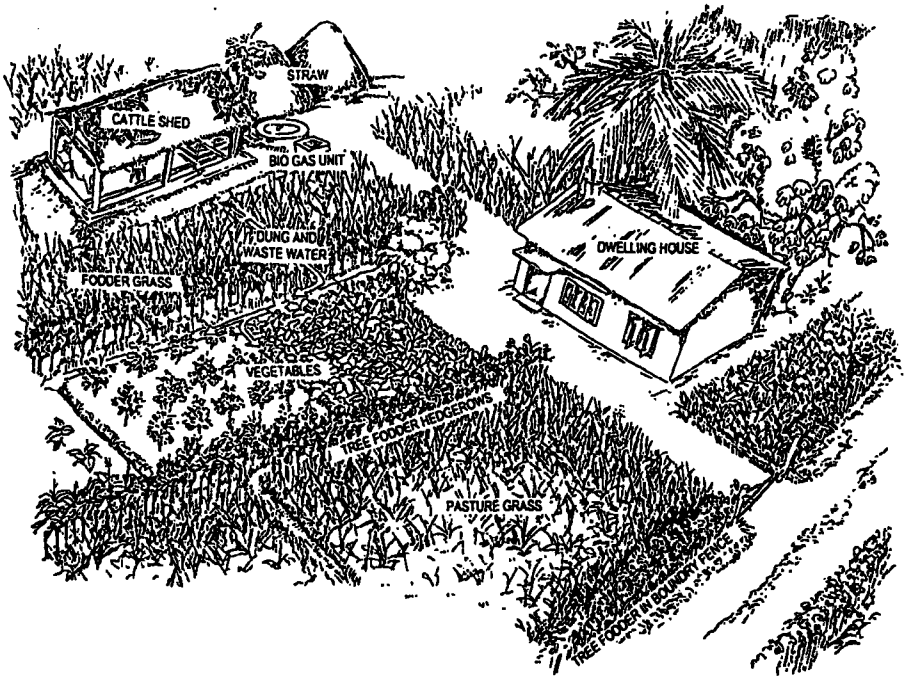


Fig. 30.1 Diagrammatic presentation of a layout for a crop-livestock integrated smallholder farm.

30.1.1 Forage along the boundary fence

Fodder trees could be established and maintained as a live fence to the maximum possible extent. Live fences reduce the cost of fence maintenance and the need to replace fence posts. The planting material is inexpensive and is available locally. e.g. Gliricidia, Ipil-Ipil, Dadap. Fodder trees along fences are commonly planted at a spacing of 0.5-1.0 m (2 or 3 ft) and harvested at a height of 1.0-2.5m (3.5 - 8 ft). Harvesting can be done at 3 month intervals. In general, 100 trees would provide enough fodder for two milking cows throughout the year. This is perhaps one of the most widespread uses of tree fodder, in small home garden systems. Live fences of Leucaena around an acre of land can produce enough fodder to satisfy 30% of the roughage intake of a cow throughout the year (Fig. 30.2).

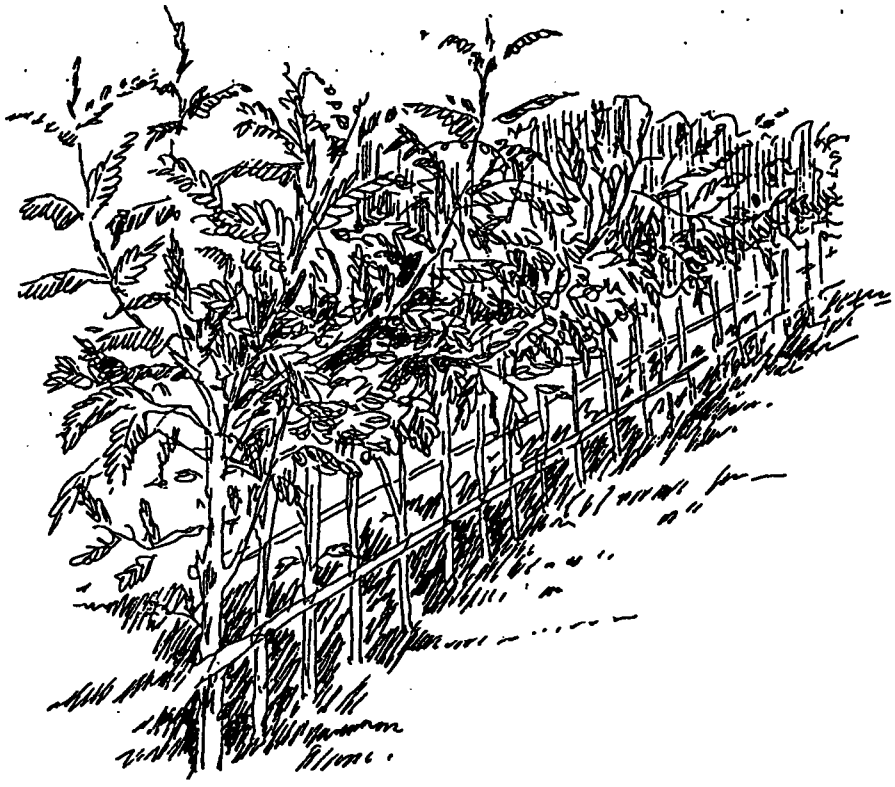


Fig. 30.2 Diagrammatic sketch of tree fodder on boundary fence

The advantages of having a live fence with fodder trees along the boundary of the home garden are many. They provide (1) shade and shelter for livestock, (2) stabilise sloping lands against soil erosion because of their deep-rooted nature, (3) supply high quality (protein rich) forage for feeding animals, (4) provide nitrogen-rich manure (green manure) for crops, (5) a source of timber and fire wood, (6) acts as a wind barrier and (7) useful as live trellises for climbing crops like pepper.

The common fodder tree species recommended for different agro-ecological zones and their yields are summarised in Table 30.1.

Table 30.1 Fodder tree species recommended and expected yields

Fodder tree species	Recommended zones	Defoliation frequency (months)	Av. fresh leaf yield kg/tree/yr
Gliricidia	MC, DZ, C, LCWZ	3 - 4	7 - 10
Leucaena (Ipil Ipil)	MC, DZ, C, LCWZ	3 - 4	5 - 6
Erythrena (dadap)	HC, MC, DZ	3 - 4	5 - 7
Albizia	MC, DZ, C, LCWZ	3 -5	4 - 6

Mid country = MC, Hill country = HC, Dry zone = DZ,
Low Country Wet Zone = LCWZ, Coconut Triangle =C

30.1.2 Forage from internal hedges and hedgerows

Hedgerow intercropping in home gardens is a practice where perennial tree legumes or shrubs are grown together with cereals crops vegetables and pasture crops. The recommended tree (shrub) species suitable for internal hedges are Gliricidia, Leucaena, Calliandra, Flemingia, Sesbania, Accacia and Erythrena.

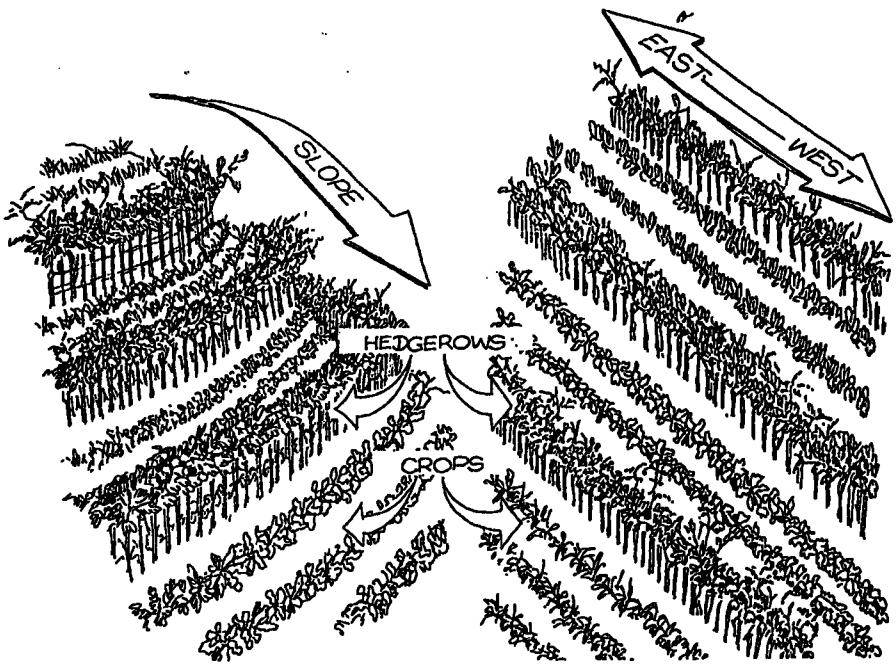
The trees are planted in wide rows and vegetable crops, cereal crops like maize or sorghum and pasture varieties are grown in the interspaces or alleys between the rows as shown in Fig. 30.3. During the cropping season the trees are pruned and the prunings are used as mulch for crops to improve the organic status of the soil and provide nitrogen to the soil. Hedges should be placed along the contour of the land or in an east-west direction to allow maximum sunlight for the crops. However, the slope of the land is the main factor that needs consideration particularly in steep/sloping lands. It is always better to plant the hedgerows along the contour to prevent soil erosion. In instances where hedges have to be placed along the slope, to allow maximum light for crops, strips of short grass (e.g. Guinea, Setaria, Vetiver) should be grown along the contour (See Fig.30.3a).

The right choice of tree species and spacing between rows and plants within rows is extremely important and to a large extent will determine the success or failure of the hedgerow farming system. There are several attributes which should be considered when selecting the tree species and spacings. In most areas, row spacing between trees ranges from 2-7 m, with 4-6 m being the commonly accepted spacing. However, the spacing may vary according to the slope of the land. Spacing of trees within rows should be as close as possible. Experience with species such as Leucaena, Gliricidia and Sesbania indicate that trees should be spaced 30 cm (1ft) apart or as near as possible to form a solid hedge along the row. This helps to obtain a favourable leaf to stem ratio, provide a more effective barrier to soil movement in sloping lands and create a better micro-environment for crop growth. Hedgerows or alley cropping is recommended for flat land as well, because it improves the productivity of the system (see Fig. 30.3b).

The advantages of the internal hedges and hedgerows are many. They (1) increase the productivity of the land due to the addition of nutrients and organic matter to the soil, (2) reduce or eliminate the use of chemical fertilisers, (3) act as physical barriers to prevent soil erosion when trees are planted in rows on sloping lands. (4) provide high quality forage and (protein rich) leaf meal for animals and (5) help weed control (due to the shade effect on the ground) and improve the appearance of the home garden.

30.1.3 Forage around the cattle shed

If there are open spaces or less shady places around the cattle shed, high yielding fodder grasses such as Napier and its hybrids. (Bana, clone-13, NB-21, Co-1, Panama etc.) can be grown as shown in Fig. 30.1. Since these grasses prefer soils with a higher content of nutrients and water, the area around the cattle shed provides a better environment. However, these grasses are not suitable in water - logged conditions.



(a) Sloping land **(b) Flat land**

Fig. 30.3 Diagrammatic sketch of tree fodder as hedgerows

30.1.4 Management of forage plants

Good management the forage plants throughout the year is essential. Appropriate harvesting practices, fertiliser application, weed control and careful management will ensure maximum forage production in the system.

30.1.4.1 *Cutting frequency (cutting interval) and cutting height (cutting intensity):* Cutting management is a very important factor that influences the productivity of tree legumes, pasture and fodder grasses in the system. Severe pruning or harvesting produces adverse effects on the subsequent regrowth (see Table 30.2 for details).

Table 30.2 Recommended cutting heights and frequencies

	Cutting frequencies (in days)	Cutting heights
Tree fodder along the main fence	75-90	4 –5 ft
Tree fodder in the internal hedges	45-60	2 ft
Grass around the shed	30	5 inches
Grass in open places	30	3 inches

It is important to remember that:

- when lopping fodder trees, a sharp knife or trimmer must be used
- frequent pruning at a lower height can minimise the shading effect of the hedgerows and reduce the effectiveness of biomass production from the hedgerow and nutrient recycling.

30.1.5 Fertiliser application

One should keep in mind that soil nutrients in the farm are constantly removed either directly through the crops like pasture and fodder, vegetables and fruits and indirectly through milk and other animal products. Hence it is vital that nutrients extracted from the soil should be replaced eventually to maintain a balance.

30.1.5.1 *Organic fertiliser*: Organic fertiliser should be used on forage plants and other crops wherever possible. It will be more advantageous to use cow dung and shed washings available on the farm, as this will help to reduce the cost on chemical fertiliser in crop production. Farm yard manure (FYM) can be used in many forms: (a) as dry solid manure, (b) as compost manure and (c) as liquid manure

Advantages of using organic manure are that:

- it increases the organic matter content of the soil
- it improves the soil structure and helps to increase aeration, water movement, microbial activity and root penetration.
- it can be applied as a mulch to the soil surface to control weed growth, conserve soil moisture and reduce soil erosion.
- it can increase the microbial population of the soil and improve soil fertility
- it can absorb excess nutrients and toxic substances
- it could be directed to a bio-gas plant to generate bio energy for household cooking and lighting.

Details of the preparation of compost and other forms of manure and their use on crops may be obtained from information leaflets and publications issued by the Departments of Agriculture and Animal Production and Health. Organic fertiliser is best applied before planting or after the fodder is harvested.

30.1.5.2 *Use of chemical fertiliser*: If the organic manure is in short supply, at the time when the need for fertiliser arises, chemical fertiliser may be used as a general mixture if and when necessary, in the ratio given below (See Table 30.3). Fertiliser application is usually practised twice a year with the onset of rains. When small plots are planted, proportionate amounts of the mixture may be applied as required.

30.1.5.3 *Weed Control:* Weeds begin to appear particularly in newly established pasture and fodder plots in the system. This can be minimised by adoption of good management practices. Regular manual weeding is the usual method of weed control in smallholder systems.

Table 30.3 Application of chemical fertiliser

Fertiliser Ingredients	Application Rate	
	kg (Ha /Yr)	kg (Ac /Yr)
Urea	200	80
Muriate of Potash	120	50
Triple Super Phosphate	120	50

1 ha=2.47 acs

30.2 Animal Housing

Housing is an essential component of an intensively managed dairy farm. Hygienic animal housing is a pre-requisite for clean milk production. However, the cattle shed need not be an elaborate and expensive construction. It can be a low cost structure, provided the basic structural features are incorporated on a scientific basis as shown in Fig. 30.4 (cattle shed for the dry, intermediate, low and mid country zones) and Fig.30.5 (cattle shed for hill country wet zone)

30.2.1 Specifications for a cattle shed (3 cow unit) recommended for the Dry, Intermediate, Mid and Low country zones (see Fig. 30.4.1 to 30.4.7 for details):

Length of shed - A shed required to house 3 cows with an enclosure for calves should be 20 feet long (add 4'3" for each additional cow).

Width of shed - The minimum width is 12' 6"

Height of shed - The minimum height of the shed should be 13' at the ridge and 8'6" at the sides.

Pillars - While the use of locally available material is encouraged, hard wood (9" diameter) is recommended. If hard wood cannot be found, concrete pillars (6" diameter) are recommended.

Roofing - It is always better to use cadjans or straw to cover the roof if the environment is hot and humid. Aluminium and zinc sheets tend to increase heat stress in animals in a hot environment.

Floor - The floor must be properly paved. The standing area of the cow and the dung channel should be made of concrete. The rest of the shed could be paved with bricks or rubble and cemented. The standing area should be 4' 9" or 5'. Floor should have a gradient of 0.5" to 2' from tie point to dung channel. Dung channel should be 12" wide and have a gradient of 0.5" to every 2' (starting from a depth of 4" sloping to 10" at the deep end).

Feeder space - The length and width of the feeder required for a cow is 4'3" x 2'

Water troughs - These could be placed in between feeders as shown in Fig. 30.4.1.

Special features - (i) a simple sprinkler system constructed with ½" PVC pipes perforated (2" apart) for spraying water over the animals during the hot part of the day (Fig. 30. 4.5).
(ii) storage space in the attic for straw and other crop residues (Fig. 30.4.6).
(iii) Hayrack for calves (Fig. 30.4.7)

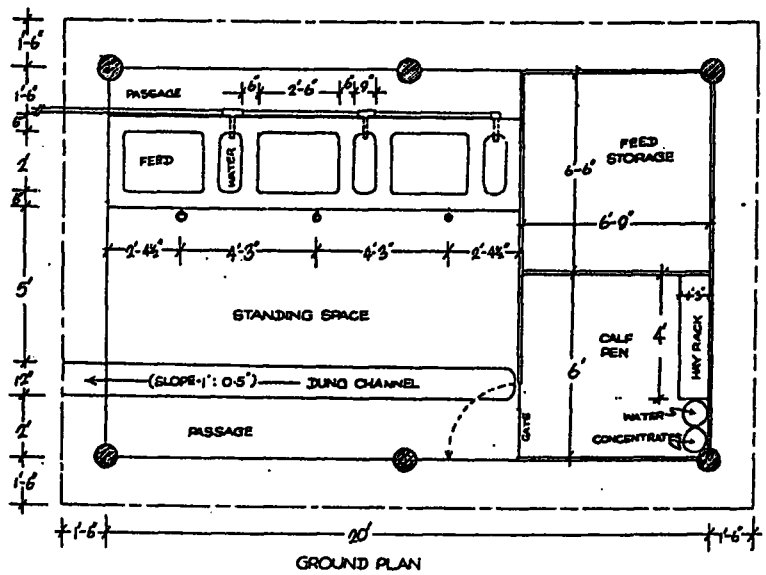


Fig. 30.4.1 Floor plan of cattle shed

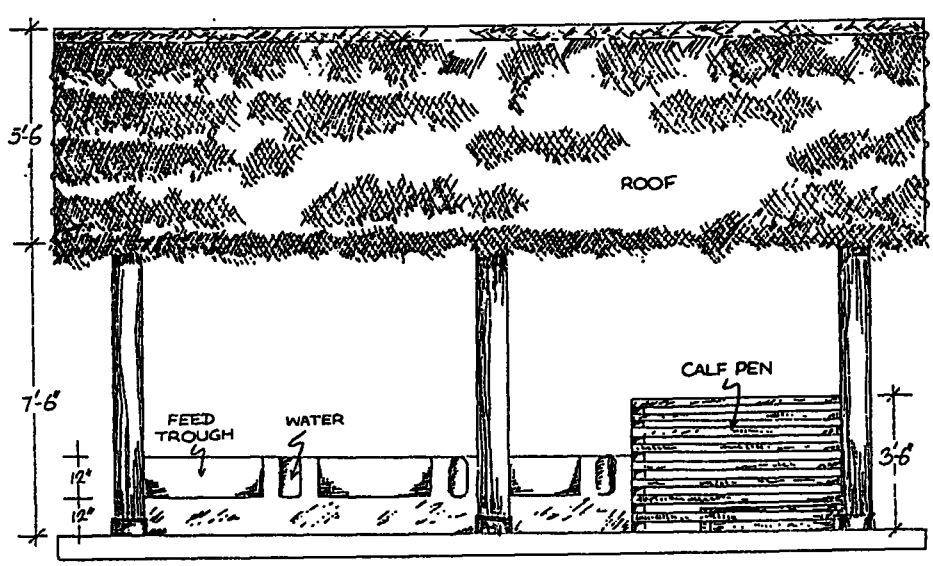


Fig. 30.4.2 Front view of cattle shed

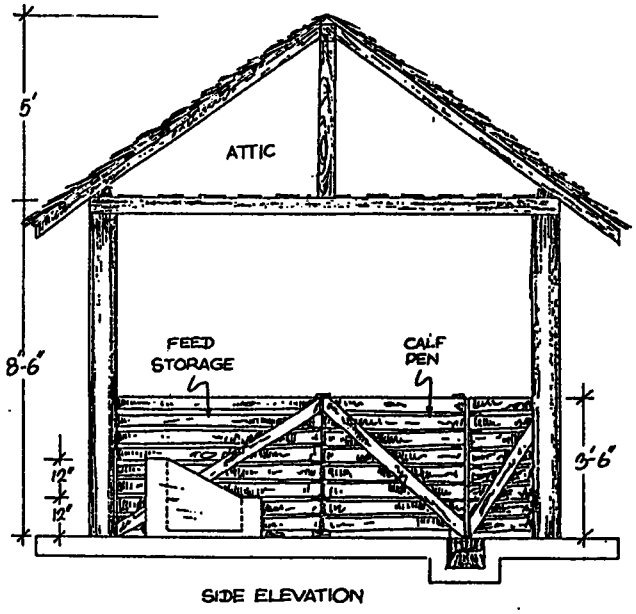


Fig. 30.4.3 Side view of cattle shed

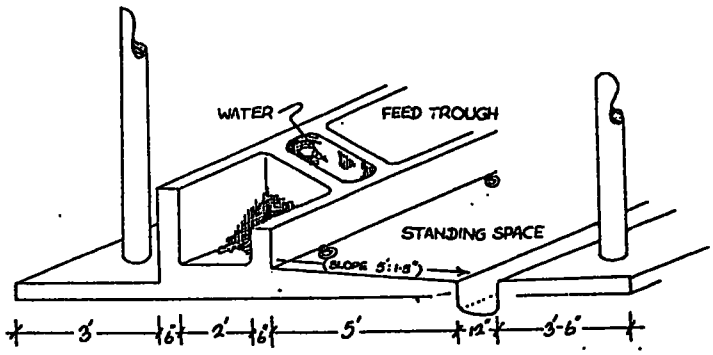


Fig. 30.4.4 Cross sectional view of cattle shed floor

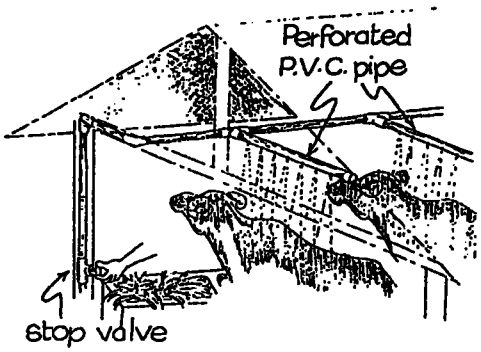


Fig. 30.4.5 Overhead sprinkler system to spray water on buffaloes

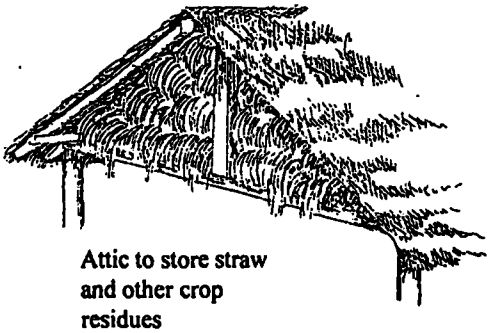


Fig. 30.4.6 Storage space in the attic for rice straw and other crop residues

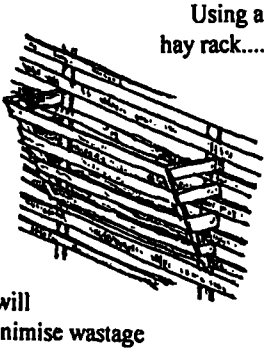


Fig. 30.4.7 Hayrack for calves

30.2.2 Specifications for a simple cattle shed (3 cow unit) recommended for the Hill country Wet zone.

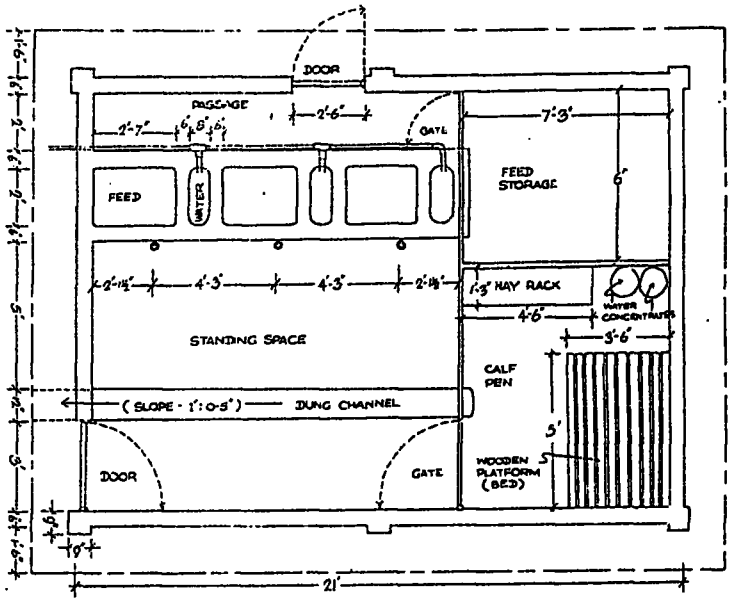
The specifications recommended earlier for the dry, intermediate and other zones need to be modified to suit colder areas in high elevations such as the hill country wet zone (e.g. Nuwara Eliya, Maskeliya, etc.) and in the cold wet areas of mid country. Modifications required for such areas are as follows:

Length of shed	-	21 feet
Width of shed	-	15 feet
Height of shed	-	The minimum height at the ridge should be 10' and 6'6" (height of pillars) at the sides.
Pillars	-	The pillars could be made of concrete (6" diameter).
Roof	-	Straw cover may be the cheapest, but needs replacement regularly. Therefore aluminium or zinc sheets are recommended. Due to the cold and wet weather prevalent in these areas, this type of roof material would not cause heat stress on the animals.
Special features:		
Boundary wall	-	Provide a 4' high boundary wall round the cattle shed to protect the animals from cold and wind. If possible cover the upper part of the wall by a wire mesh (1"x1"). During very cold weather, this area may be covered by suitable material (e.g. gunny bags, polythene).

Door - As the cattle shed is enclosed on all the sides, a door of 6'x3' should be provided on one side. Provide a door at the back to enter the feeding passage and the feed storage area and a gate for the calf pen (see Fig 30. 5.1).

Calf pen - Provide a slatted wooden platform 5' x 3'6" for the calves as a sleeping area as the cement floor can be damp and cold. The platform should be raised 3" - 4" from the floor and should also be movable for cleaning the floor.

Diagrammatic sketches of the floor plan, lateral and frontal views and cross sectional view of the floor of the cattle shed recommended for the hill country wet zone are given in Fig. 30.5.1 to 30.5.4.



30.5.1 Floor plan

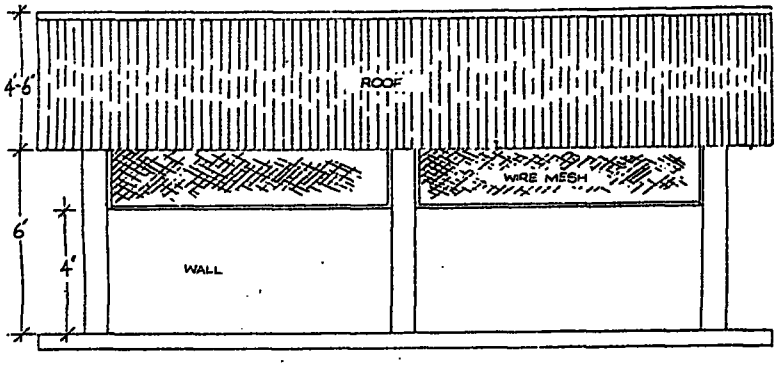


Fig. 30.5.2 Front view

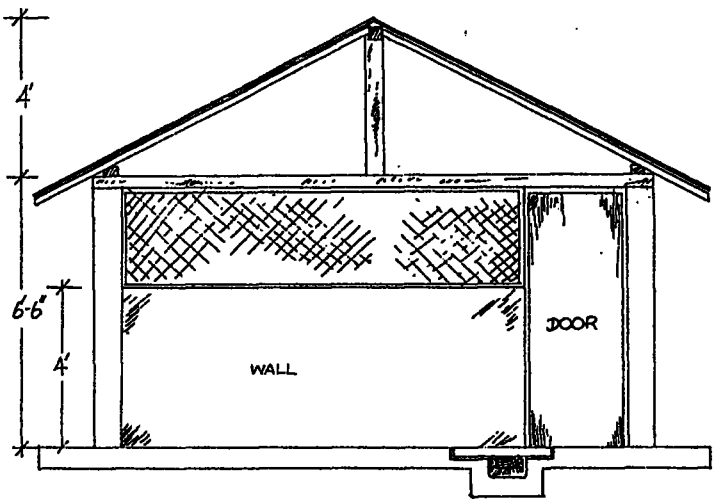


Fig. 30.5.3 Side view

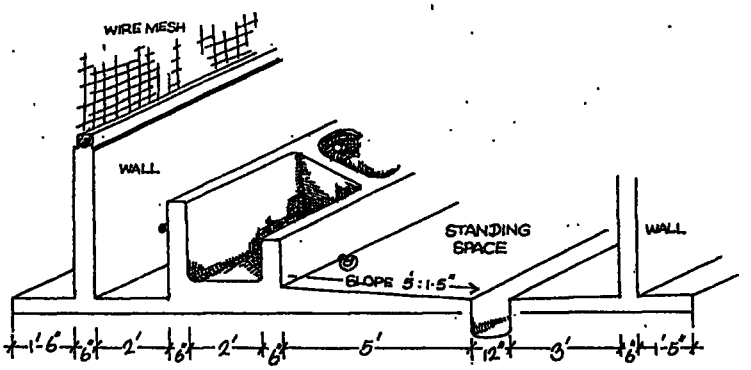


Fig. 30.5.4 Cross sectional view of the cattle shed floor

30.2.2.1 *Locating the shed:* The cattle shed should preferably be sited at a relatively high elevation in the farm or homestead, so as to facilitate the use of cattle manure, urine and shed washings to fertilise the fodder and vegetable plots through gravity irrigation. This could be achieved through a system of well designed, simple drains originating from the cattle shed (see Fig. 30.1)

30.2.2.2 *Cleaning and maintenance of the shed:* The shed should be cleaned twice a day prior to milking. The animals should also be washed and cleaned at the same time. Soiled animal bedding and left over roughage feed should be collected and put away in a separate place or put into the compost pit before washing the shed. The dung and urine should be pushed out through the dung channel. Subsequently the shed should be washed, cleaned and the washings directed to the dung pit away from the shed and or to a bio-gas plant. The dung may be recycled as raw material for compost, for generation of biogas and in other ways described elsewhere.

30.3 Management of the animals

Good management of animals is a prerequisite to an efficient farm operation. Management encompasses several activities such as (i) handling and care of animals, (ii) feeding, (iii) milking, (iv) reproduction and breeding, (v) health care and (vi) maintenance of records. Detailed descriptions on the above activities have been given in other chapters.

In this Chapter emphasis has been given to important subjects that have not been included in the others. It has also provided information on some of the more important topics relevant to crop/stock integration in summary form, though some of these aspects have been discussed elsewhere.

30.3.1 Handling and care

Animals need love and care, and when these are provided, they operate at maximum efficiency. Therefore, herdsman or farmer must have a friendly relationship with the animals and handle them with gentleness at all times. The place where animals are kept must be properly ventilated and provided with good lighting. During the hot part of the day, bathing of animals will facilitate reduction in body temperature. This is particularly important with buffaloes whose body cooling mechanism is not very efficient. That is the reason why they need to wallow in water during the hot part of the day. If buffaloes are reared indoors, sprinkling or splashing of water over the body at 1-2 hourly intervals 3-4 times a day during midday is very desirable.

30.3.2 Feeding and provision of water

Dairy cows and buffaloes must be fed adequately to obtain optimal milk and meat production. In general, they must be given grass and fodder *ad libitum*. The requirement is 10% of the body weight on wet weight basis. The limitations, and method of overcoming these limitations in ruminant feeding have been described in Chapters 1,2,3,4,5 and 6. Grass and fodder should be available in sufficient quantities always in the feed trough, so that animals could feed during the day and night. If grass and fodder are poor in quality and not available in sufficient quantities, they must be supplemented with tree fodder, or a suitable feed supplement such as concentrate feed or UMM feed supplement. Animals must be fed to satisfy both the maintenance and production requirements. Practical guidelines on the appropriate levels of feeding are given in Chapter 6.

Provision of an adequate supply of clean drinking water throughout the day is of paramount importance. Thirsty stock do not eat as much food as those that which are well watered. An average cow (200-300 kg) requires about 30-40 litres of water (15% of the body weight) for maintenance and an additional 4-5 litres for each litre of milk produced. It is best that animals should have access to clean drinking water throughout the day, and ideally it could be provided in a built - in water trough within the feed trough as shown in the diagram given in this chapter. The water trough could be filled *via* a storage tank of a reasonable size, constructed at the end of the shed and fitted with a simple ball valve device. As the water level in the trough goes down, it will be

filled automatically through a gravity.

30.3.3 Milking and clean milk production

The ultimate aim of dairy farming is to produce milk and market it as a safe human food. Therefore, hygienic practices with regard to milking is of paramount importance. In almost all smallholder operations, cows are milked by hand. Cows are usually accustomed to a stimulus for milk let-down. In zebu animals and indigenous buffaloes, milk let-down is stimulated by the sight of the calf and/or suckling by the calf. With most dairy breeds of cattle and buffaloes, the routine milking procedures such as the time of milking, washing the udder with warm or cold water, the noise of the milking bucket, feeding of concentrate, etc. are adequate stimuli for milk "let-down". Milk removal is done by 3 methods:

(a) Strip milking - where the teat is held between the thumb and first finger and milk is squeezed out by pulling the hand down along the teat,
(b) Pinch milking – where the teat is pinched and stripped rhythmically, holding the teat between thumb and middle fingers and
(c) Rhythmic squeezing - In this method the teat is rhythmically squeezed by encircling the upper part of the teat with the thumb and first fingers while keeping the remaining fingers in close contact. The milk is squeezed out by tightening the fingers starting with the index finger, followed by middle and then little fingers. All fingers are relaxed simultaneously and a new squeezing sequence begins (Fig. 30.6). This method is the most preferred method as it causes no damage to the teat, compared to the first two methods.

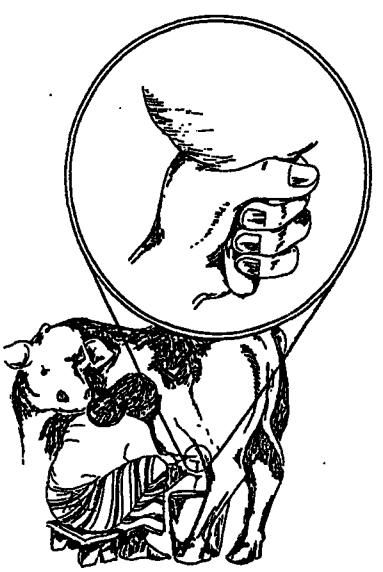


Fig. 30.6 Recommended method of milking - rhythmic squeezing

The milk collected from the teat must be free from contamination with pathogenic organisms as the milk would spoil within a short period of time. Hence the following procedure is recommended in milking of cows.

purpose.

(d) Milk must be transported in clean bulk containers (5 or 10 litre cans) and this should be done without delay.

30.3.4 Reproduction and breeding

Heifers must be bred at the first detected heat if they have reached sufficient body weight or size (approximately 2/3 of the mature body weight). Cows should be bred at the first or second oestrus which must occur within 60 days postpartum. If the first postpartum oestrus occurs within 30-45 days postpartum, skipping the first oestrus is recommended as the chances of conception are very low around this period. The cow should be examined for oestrus at least 2-3 times a day, in addition to milking times. The cow should be inseminated between 10-18 hours after the onset of oestrus to obtain an optimum conception rate. The cow should be examined again for oestrus, more frequently between18 to 22 days after the service and if the animal has not returned to oestrus, it should be examined for pregnancy between 65 to 90 days after the service. Any cow that fails to conceive after 3 repeated inseminations must be checked and necessary advice and treatment provided. The goal should be to get the cow pregnant within 90 days post-calving and thereby get one calf every year. The right type of bull or semen to be used for mating a cow or buffalo depends on the genotype of the animal and the purpose and method of rearing which will vary with the agro-ecology of the region.

30.3.5 Health care of the animals

The economic efficiency of a farm depends very much on the productivity of the animals and the health status . Detailed information on matters relating to health is given Chapters 18 to 21 and Chapter 29 in this Handbook. However, a brief summary of the important aspects of health care and disease prevention are given here.

Health of an animal is expressed by its appearance and physical activity. A shining skin coat, alertness (i.e. quick response to stimuli), normal stance and gait and good appetite always signify good health. Any departure from these usually suggest an illness. As some diseases are preventable, a programme for routine vaccination and worming are given in Chapter 29 of this Handbook.

30.4 Utilization of animal waste on farm crops

Cattle and buffalo dung and urine (farm yard manure) is a good source of organic nutrients for the soil and plants. This material is rich in nitrogen, phosphorus and potassium. The proportion of nitrogen, phosphorus and potassium in farm yard manure is around 2, 0.4 and 1.7% respectively. Research studies indicate that the availability of nitrogen, phosphorus and potassium in farm yard manure is as follows: 1/3 of nitrogen, ½ of the phosphorus and most of the potassium required for vegetable crops. On the other hand 100 kg of farm yard manure is equivalent to 15-18 kg of urea, 12-15 kg of Triple Super Phosphate and 15-18 kg of Muriate of Potash. Faeces and urine when mixed will form an excellent organic fertilizer. Therefore animal dung (or slurry) could be applied to crops thereby reducing the need for as well

as the cost of fertilizer when inorganic manure is used. Besides, farm yard manure can also be recycled through a bio-gas plant to generate energy for domestic cooking and lighting at a relatively low cost.

Selected references

Abeygunawardena, H., Siriwardene, J.A. de S. and Subasinghe, D.H.A (1998) (eds.) *Cattle and Buffalo Farming - A Training Manual for extension workers.* Colombo, Sri Lanka. National Science Foundation Press. 113 pp.

Carlos E. Laseano and Werner, S.W. (1998). Small Farm Production Systems for Ruminant Animals in Tropical Regions. In: *Proceedings 8th World Conference on Animal Production,* Symposium Series-1, South Korea, pp. 377-391.

De Jong, R., Kuruppu, L.G., Jayawardena, Q.W. and Ibrahim, M.N.M. (1994) Performance of small livestock crop demonstration farms in Sri Lanka. *Asian-Australasian Journal of Animal Science,* 7, 571-582.

Devendra, C. (1996) Overview of integrated animals -crops-fish production systems: Achievements and future potential. In: *Proceedings Symposium on Integrated Systems of Animal Production in the Asian Region,* Chiba, Japan, pp. 9-22.

Devendra, C. Thomas, D. Jabbar, M.A. and Kudo, H. (1997). *Improvement of Livestock Production in Crop-Animal Systems in Rain fed Agro-ecological Zones of South East Asia.* International Livestock Research Institute (ILRI), Nairobi, Kenya. pp. 116.

Gutteridge, R.C. and Shelton, H.M. (1994) (eds.) *Forage Tree legumes in Tropical Agriculture.* CAB International, Wallingford, Oxon OX10 8DE, UK.

Ibrahim, M.N.M. (1998) *Feeding tables for ruminants in Sri Lanka,* Peradeniya, Sri Lanka.

Markham, J.P. and Malcolm, L.R. (1986) (eds.) *The economics of tropical farm management.* London, Cambridge University Press. pp. 178.

Pasture Research Committee (1982) *A guide to the establishment, maintenance and utilization of forage plants.* Ministry of Rural Industrial Development, Colombo, Sri Lanka.

Perera, B.M.A.O., Ranawana, S.S.E., Fernando, S.T., Jalatge, E., Kuruwita, V.Y., De Alwis, M.C.L. and Weilgama, D.J. (1998) (eds.) *The Sri Lanka Water Buffalo.* Science Education Series. No. 31, Colombo, Sri Lanka, NARESA Press. 74 pp.

Perera, B.M.A.O, Siriwardene, J.A. de S., Horadagoda, N.U. and Ibrahim, M.N.M. (1996) (eds.) *The Role of the buffalo in rural development in Asia.* Colombo, Sri Lanka.-NARESA Press. 522 pp. ISBN 955 - 590 - 007 - 8.

Subasinghe, D.H.A., Horadagoda, N.U., Abeygunawardena, H and Siriwardene, J.A. de S. (1998) [eds.] *Water Buffalo-Improved Utilisation through New Technologies.* Colombo, Sri Lanka. National Science Foundation Press. 94pp.

Wijewardena, R. and Waidyanatha, P. (1984) (eds.) *Conservation Farming for small farmers in the humid tropics.* Manuel published jointly by the Department of Agriculture, Sri Lanka and Commonwealth Consultative Group on Agriculture for the Asia Pacific Region.