Tropical Agricultural Research and Extension 3 (1): 75 - 81, 2000

Use of milk progesterone RIA to determine factors affecting the success rate of artificial insemination services in cattle in mid-country small-holder farms in Sri Lanka

H. Abeygunawardena,[1] P.A.B.D. Alexander,[1] I.S. Abeygunawardena[2] & B.M.A.O. Perera[1]
[1]Department of Veterinary Clinical Studies, University of Peradeniya, Peradeniya, Sri Lanka
[2]Department of Veterinary Pre-Clinical Studies, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Peradeniya, Sri Lanka

ABSTRACT

A measurable quantity of progesterone in a blood or milk sample signifies the presence of luteal tissue in the ovary. Thus progesterone concentration in blood or milk samples collected at strategic intervals when interpreted in the light of other information is of diagnostic value in fertility investigations in cattle. Therefore, a study was conducted using milk progesterone RIA as a tool to monitor the efficiency of AI services and to identify factors affecting its success rate. Milk samples were collected on days 0 (day of AI), 10-12 and 20-24 from dairy cows bred by AI in five randomly selected VS ranges in wet zone, mid country region of Sri Lanka. Information relating to the farm, the cow, the bull/semen and the inseminator was recorded, together with repeat service dates. Pregnancy diagnosis was done by rectal palpation at 60-90 days. Progesterone values on day 0 (n=206) showed that the concentration was high at the time of AI in 17% of cows, indicating that they could not have been in oestrus. Progesterone values of two samples collected on day 0 and 10-12 (n=182) showed that only 57.1% of the animals had normal ovulatory cycles while 13.2% were either in anestrus or had anovulatory oestrus or had short luteal phases. Results of examination of cows with all three samples (n=110) showed that 61.8% conceived, 10.9% had non-fertilization, embryo mortality or persistent CL, and 5.5% had been inseminated during pregnancy. The overall CR varied significantly ($p<0.05$) between locations from 33.8% to 61.4%. The most significant limiting factor which influence the success rate of AI appears to be the poor heat detection by the farmers coupled with other factors such as timing of AI in relation to onset of oestrus, quality of semen and competency of technicians.

Keywords: Artificial insemination, cattle, progesterone, radioimmunoassay.

INTRODUCTION

Development of the radioimmunoassay (RIA) technique in the 1960s provided a very sensitive, precise and robust method of quantifying the minute amounts of hormones present in a variety of biological fluids such as blood, milk and urine. In the field of livestock production and reproduction, it is a valuable research tool, a diagnostic aid and a method of monitoring responses in biotechnological and other applications (Perera and Abeyratne 1979; Dargie and Perera 1994). The use of RIA techniques for studies on livestock production in Sri Lanka commenced in the 1970s and has been applied mainly in buffaloes and cattle, as a method of characterizing reproductive pattern of water buffalo (Abeygunawardena *et al.* 1996), monitoring responses to oestrous synchronization (Perera *et al.* 1978; Rajamahendran and Thamotharam 1983; Perera *et al.* 1987), for studying causes of reproductive failure (Perera *et al*.1984; Perera *et al.* 1987), for early pregnancy diagnosis (Perera *et al.* 1980; Perera 1986), to determine accuracy of oestrous detection (Mohamed *et al.* 1990; Abeygunawardena *et al.* 1995a), and for monitoring postpartum ovarian activity under different suckling management practices (Mohan *et al.* 1990; Perera *et al.* 1992).

The progesterone concentration in blood or milk samples collected at strategic intervals, when interpreted in the light of other information from physiological, clinical and visual observations, is a valuable indicator of the reproductive status of the animal and is of diagnostic value in the identification of some of the factors which may have affected the outcome following a service by a bull or an artificial insemination (Garcia *et al.* 1995). In farm animals, progesterone is secreted by a transitory structure of ovary called CL formed from the ovulated follicle of the preceeding oestrus. In cattle and buffaloes, the

Abbreviations: RIA - radioimmunoassay, AI - artificial insemination, VS - veterinary surgeon, CL - corpus luteum, MSL - mean sea level, CR - conception rate, CFSI - calving to first service interval, CCI - calving to conception interval, S/C - services per conception.

CL remains functional for a variable period of time depending on the presence or absence of a conception; that is for 260-280 days if a conception has occurred and for only 15-16 days in the absence of a conception (Peters and Ball 1995). Thus a measurable quantity of progesterone in a blood or milk sample signifies the presence of luteal tissue, such as in the luteal phase of the cycle and pregnancy while the absence of detectable levels of progesterone indicates the absence of luteal tissue such as in oestrus or in anestrus and this forms the basis for the use of RIA of progesterone for diagnostic purposes.

In Sri Lanka, AI has been considered as the primary tool to improve the productivity of the existing cattle and buffalo populations (Livestock Development Strategy 1988). The Sri Lankan dairy industry is characterized by the several hundred thousands of smallholder farmers and a few cooperate processors. The smallholder dairy farms account for more than 95% of the milk produced in the country and for more than 90% of milk collected by the formal milk collecting channels (Soni *et al.* 1991). Though AI was first introduced into the country as a field service in early 1950s, even after 40 years its coverage is still limited to about 15% of the breedable cattle population and accounts for less than 5% of estimated annual calvings (Sri Lanka Livestock Statistics 1991/92; Abeygunawardena and Amarasekera 1995). This technique has been widely used in the wet zone region which carries about a quarter of the national cattle population and has a high percentage of *Bos taurus* blood types together with favourable climatic and infrastructural facilities (Soni *et al.* 1991; Mohamed *et al.* 1990; Abeygunawardena and Amarasekera 1995). However, the average first service conception rate achieved in this region appears to be less than 35% and repeat breeding is a major cause of infertility in cattle subjected to AI (Mohamed *et al.* 1990; Abeygunawardena *et al.* 1995a). This study therefore, was undertaken to determine the factors relating to the cow, the farm, the technician and the semen which may affect the success of AI in smallholder farms in the wet zone, mid-country region of Sri Lanka.

MATERIALS AND METHODS

Field Study

The areas (ranges) covered by five government VS ranges which were subjected to a previous questionnaire survey relating to AI (Alexander *et al.* 1997), namely Gampola, Kundasale, Teldeniya, Udunuwara and Yatinuwara, were selected for the study. These areas adequately represent the mid-country smallholder farming systems and are located at elevations between 500-1,000 meters above MSL The farming system is characterized by rearing a small herd of dairy animals (herd size ranges from -4 animals per smallholding) managed intensively (i.e. cut and feed system without any grazing) or semi-intensively (i.e. Cut and feeding with tethered grazing).

Sampling Protocol

The sampling protocol outlined below was based on the recommendations of the International Atomic Energy Agency in Vienna, which is sponsoring a co-ordinated research programme with participation from 12 countries in Asia and Latin America. The study is on-going and the results presented here relate to the period January 1996 to August 1997.

The study was conducted in liaison with the range VSs and the AI technicians serving these ranges and farms. Dairy cows receiving first inseminations following a recorded calving were monitored until they were confirmed pregnant. One of the investigators accompanied the inseminator and, at the time of AI (day 0), recorded detailed information relating to the farm (*i.e. land extent, herd size and composition, management system, income from livestock and other sources, time spent on dairy farming and breeding practices*), cow (*i.e. cow identification, date of birth, parity, last calving date and type, date of first postpartum oestrus, lactation stage, body weight and body condition, milk yield, code of semen, time of the day of AI, visually appreciable oestrous signs and degree of uterine tone*), semen (*i.e. breed of semen donor, bull identification number, semen source and type, semen batch, semen dose and quality of semen as a percentage of motility*) and the inseminator *(i.e. age, years of experience, type of employment and site of semen deposition)*. A milk sample (10 ml) was collected in to a bottle containing a tablet of potassium dichromate as a preservative. The investigator returned to the farm between 10-12 days post-service to collect a second milk sample and again between 20-22 days post-service to collect the third milk sample, if the cow was not presented for a repeat AI. Repeat service dates were recorded for cows returning to service and those not returning to oestrus were examined for pregnancy by rectal palpation, 60-90 days after the last AI. A total of 384 cows from 384 smallholdings were covered during the study period and the number of cows entered into different estimates varied with the parameter as there have been missing data for some entries. The number of cows or observations taken into consideration for estimation of each parameter are presented in parenthesis in results section.

Milk Progesterone Assay

Milk samples were placed in a refrigerator (4°C) within 6 hours of collection and transferred to the laboratory for processing within 7 days. They were centrifuged at 4°C at 1000 rpm for 10 minutes, the fat-free fraction (skim-milk) was drawn off and stored at -15°C until assay. Progesterone concentration was determined using RIA kits supplied by the Joint FAO/IAEA Division, Vienna (Alexander *et al* 1997). The assay was a solid-phase technique employing antibody-coated tubes, ^{125}I-progesterone as tracer and standards prepared in skim-milk. The intra-assay and inter-assay coefficients of variation were 9% and 14.5%, respectively.

Data Tabulation and Analysis

The data were entered into the Artificial Insemination Database Application (AIDA) developed under Microsoft Access and supplied by the Joint FAO/IAEA Division. Means and frequencies (as percentages) were calculated and the summary data were examined for trends and associations among estimates of parameters and variables. The ANOVA was performed whenever appropriate.

RESULTS

Progesterone Data Interpretation

The progesterone values in milk ranged from < 0.5 nmol/l (undetectable) to 35.0 nmol/l. Any value above 3.0 nmol/l (high) was considered as indicative of CL function, whereas any value less than 1.0 nmol/l (low) was considered as indicative of absence of luteal function. Values in the intermediate range (1.0 - 3.0 nmol/l) were considered as inconclusive.

Results from milk progesterone assay for the first sample collected on the day of AI (day 0, n=206) revealed that 75.2% of the AIs were performed when progesterone was below 1 nmol/l, indicating that the cow could have been in heat, but 17% of AIs were done when progesterone was above 3 nmol/l, when the cow could not have been in heat and was most likely in the luteal phase (Table 1). The remainder of

Table 1. Diagnosis based on progesterone concentration in first sample only (Day 0, day of AI).

Progesterone on Day 0 (AI)	No. of Observations and Percentage of Occurrence*	Interpretation
Low	155 (75.2)	Progesterone concentration below 1.0 nmol (negative range) indicates that AI has been performed at a time other than the luteal phase (i.e. may be during oestrus).
High	35 (17.0)	Progesterone concentration above 3.0 nmol/l (positive range) indicates that AI has been done during the luteal phase (i.e. during inappropriate time).
Intermediate	16 (7.8)	Progesterone between 1-3 nmol/l (inconclusive range) should be correlated with other physiological and clinical data, and may indicate that AI was done too early or too late relative to oestrus (i.e. during inappropriate time).
Total observations	206 (100)	

* Values are given in parenthesis.

Table 2. Diagnosis based on progesterone concentration in first two samples.

Day 0 (AI)	Day 10-12	No. of Observations and Percentage of Occurrence*	Interpretation
Low	High	104 (57.1)	Progesterone concentration within negative range of day 0 and within positive range on day 10 indicates and ovulatory cycle.
Low	Low	24 (13.2)	Progesterone concentration within negative range on both days indicates anoestrus, anovulation or short luteal phase, which may be more frequently found in animals which were in anoestrous before AI.
High	High	14 (7.7)	Progesterone concentration within positive range on both days indicates AI on pregnant animal or luteal cyst.
High	Low	14 (7.7)	Progesterone concentration within positive range on day 0 and within negative range on day 10 indicates that AI was performed during luteal phase, if next heat occurs after 7-14 days.
*	*	26 (14.3)	At least one of the samples showed an intermediate value (1-3 nmol/l). Other clinical data is required fro proper interpretation.
Total observations		182 (100)	

the samples (7.8%) had values in the inconclusive range (1-3 nmol/l), indicating that AI might have been done either too early or too late in relation to standing oestrus. Progesterone values of the first two samples, collected on days 0 and 10-12 (n=182) showed that 57.1% of the animals had normal ovulatory cycles, while 13.2% were either in anestrus, had anovulatory oestrus or short luteal phases (Table 2).

The progesterone results from all three samples (days 0, 10-12 and 21-23) together with findings from rectal palpation for pregnancy diagnosis were available for 110 cows (Table 3). In this sub-sample, 61.8% of the cows conceived to the first service, 6.4% had non-fertilization or early embryo mortality, 4.5% had late embryo mortality or

Table 3. Diagnosis based on progesterone concentration in all three samples.

Day 0 (AI)	Day 10-12	Day 22-24	Pregnancy Diagnosis	N of Observations and Percentage of Occurrence*	Interpretation
Low	High	High	Positive	68 (61.8)	Pregnant
Low	High	Low	Negative	7 (6.4)	Non-fertilization, early embryonic mortality or post-AI anoestrus.
Low	High	High	Negative	5 (4.5)	Late embryonic mortality (> day16), luteal cyst or persistent CL.
High	High	High	Positive	6 (5.5)	AI on pregnant animal.
*	*	*	Positive or Negative	24 (21.8)	At least one of the samples showed an intermediate value (1-3 nmol/l Other clinical data is required for proper interpretation
Total Observations				110 (100)	

* Values are given in parenthesis.

Table 4. Fertility indices in cattle subjected to AI in four selected veterinary ranges in the mid-country of Sri Lanka.

Veterinary Range	Means (± SD) interval (days) from calving to:		Conception rate (%) to:		Services per Conception
	First service*	Conception*	First service	All services	
Gampola	130±51.5 (n=34)	112±50.6 (n=16)	50.0 (n=34)	50.0 (n=34)	2.0
Kundasale	170±114.0 (n=52)	187±74.8 (n=39)	34.6 (n=52)	45.9 (n=85)	2.2
Teldeniya	137±272.1 (n=53)	166±105.0 (n=22)	30.4 (n=56)	33.8 (n=65)	2.9
Udunuwara	186±81.1 (n=83)	206±97.9 (n=72)	53.6 (n=48)	61.4 (n=127)	1.6
Yatinuwara	242±103.3 (n=45)	278±120.6 (n=15)	33.3 (n=45)	35.2 (n=54)	2.8

* Calving to conception interval was calculated from a sub-sample of the data set used for calculating the calving to first service interval.

persistent CL, and 5.5% had been inseminated during pregnancy.

Fertility Parameters of Cows Subjected to AI

The fertility indices for the cows in this study are given in Table 4. The overall average calving to first service interval (CFSI) was 164.8±138.8 days (n=348) and the calving to conception interval (CCI) was 196.2±100.7 days (n=174). The average CFSI in the different VS ranges varied from 130 days at Gampola to 242 days at Yatinuwara. The CR to first service and to all services varied from 30.4% and 33.8% respectively at Teldeniya, to 53.6% and 61.4% respectively at Udunuwara. The overall S/C ranged from 1.6 at Udunuwara (n=127) to 2.9 at Teldeniya (n=65).

Effect of Different Factors on Success of AI

Farm factors: No significant differences in CRs were evident in animals managed semi-intensively and intensively. However, higher CRs (41.1%; n= 129) were observed in animals maintained intensively and fed concentrates than those managed semi-intensively and fed concentrates (36.0%; n=239), those managed intensively but not fed concentrates (33.3%; n=9), or grazing only (14.3%; n=7). Those with less than 25% of the family income from livestock had CR of 23.4% (n=111), while those with higher proportions of income from dairying had CRs above 30% (n=217). Similarly, for AIs performed 6, 12, 18, 24 and 30 hours after first detection of heat, the CRs were 20% (n=100), 32.1% (n=109), 33.3 (n=93), 52.0% (n=52) and 37.5% (n=8), respectively. Higher conception rates (>35%) were recorded when the farm was less than 10 km from the AI station.

Cow factors: The CR was above 35% up to the third parity but declined thereafter. Cows receiving their first service before 60 days postpartum had lower CR (<20%) than those receiving the first service after 60 days (> 25%). Effects of other cow factors *(i.e. age, type of calving, lactation stage, body weight, body condition, milk yield, time of the day of AI, visually appreciable oestrous signs and degree of uterine tone)* were inconclusive.

Bull/semen factors: Semen from 22 bulls had been used in the sample under study. Of these, semen from seven bulls had been used for more than 20 services and their CRs ranged from 18.2 to 70.4%. Semen originating at the Kundasale AI station gave higher CR (49.7%, n=188) than imported semen (31.6%, n=263). Similarly, locally processed chilled semen recorded higher CR (43.2%, n=37) than imported or locally processed frozen semen (38.6%, n=414).

Inseminator: The CR achieved by the different technicians ranged from 32.3 to 58.9%, confirming a significant technician effect ($P<0.05$). All technicians monitored had more than 2 years experience in AI and were also engaged in work other than AI. Government employed AI technicians fared better (CR of 54.1%; n=218) than self-employed AI technicians (CR of 37.4%; n=91).

DISCUSSION

In this study, an attempt was made to collect information on a large number of factors related to the performance and outcome of AI services in small-holder farms. However, poor record keeping by the farmers, heterogeneity of genotypes maintained in small-holdings and subjectivity of the assessment of some factors resulted in insufficient data being available for some of the parameters to allow accurate interpretation. Further, several of the factors were interrelated and therefore produced confounding effects. However, a few factors which were perceived to be of practical importance were found to have an overriding influence on the outcome of AI as reflected in CR. These included: heat detection efficiency; management and feeding; timing of insemination; distance from AI unit to farm; semen source and type; and AI technician. These provide insights to ways in which the fertility of cattle in this region, as well as the AI service, might be improved.

As evidenced from milk progesterone profiles, the efficiency of oestrous detection by the farmers was poor. Around 20% of the animals presented for AI had high progesterone levels and therefore could not have been in heat. Even in those with low progesterone levels at the time of AI, only about 57% appeared to have had a normal ovulatory oestrus. These results reinforce the findings of a previous study (Mohamed *et al.* 1990; Abeygunawardena *et al.* 1995a) and indicate the need for farmer education and training in the reproductive management of dairy cattle.

In animals managed intensively or semi-intensively, higher CR was found in those fed concentrates as a supplement to roughages than in those not fed concentrates. The effects of nutrition on reproduction and fertility have been well documented (Peters and Ball 1995). Improved nutrition will not only reduce the postpartum anoestrus period, but will also improve CR. The finding that farms with higher proportions of their total family income from dairying had higher CR than those with lower proportions indicate that these families attached more importance, and hence paid more attention, to the management of their cows. Presumably, this included better feeding and heat detection, resulting in higher fertility.

The average calving to first service interval in this study was 165 days, whereas in a previous study in this area (Abeygunawardena *et al.* 1995b) the average interval from calving to first rise in plasma progesterone was around 75 days. Although poor nutrition could have prolonged the period of postpartum anoestrus as suggested by the authors, in a proportion of cows in the present study, it is likely that even those which returned to oestrus early in the postpartum period were kept unbred for an extended period. This is one of the major reasons for the prolonged calving to conception interval as shown in this study.

The overall CR ranged from 33.8% to 61.4% in the sample of VS ranges studied. While in three ranges it was at the lower limit of the acceptable range, in two ranges it was below the acceptable limit. Considering the low AI coverage of the breedable cattle population in the wet zone area, this level of success is unlikely to have any measurable impact on diary cattle productivity. Of the five VS ranges studies, two VS ranges recorded conception rates above 50% with one recording 61.4%, the other three recorded CRs below 50% with one VS range recording CR as low as 33.8%. As the infrastructural facilities, climatic conditions, genotypes of animals, semen source do not vary much among the VS ranges studied, these observed differences in conception rates are most likely to be attributable to the factors relating to the farm (eg. heat detection) and inseminator (eg. technical competence and motivation). Thus it is very conceivable that any attempt to improve the performance of AI services should focus on training of farmers to improve the efficiency of heat detection and continuing education programmes for AI technicians coupled with regular monitoring of the performance of technicians for improving their knowledge, skills and motivation.

The timing of insemination relative to first detection of heat is known to be critical for achieving high CR (Peters and Ball 1995). In the present study the CR increased as the interval increased from 6 up to 24 hours, and then declined. In theory, the optimum time for service is 12-18 hours after onset of "standing" heat. However, in the present study, the animals were housed or tethered and there was no opportunity for them to exhibit standing heat. Thus it is likely that the farmers' reliance on signs such as vaginal mucous discharge and bellowing may have resulted in animals being detected during pro-oestrus, several hours before the actual onset of oestrus. This could explain the above results, since AI done 18-24 hours after detection by the farmer would mean that AI was in effect could have been done 12-18 hours after onset of standing heat.

The distance from the AI unit to the farm also had an influence on the success rate. This is likely to be due to farmers residing close to the VS office having easier access to AI technicians compared to those that are more distant, as well as to technicians possibly giving priority to AI calls from nearby farms than to those from distant ones. Therefore the cows in smallholdings located closer to the AI service point may be receiving services in time compared to those located in distant places. Thus delays in obtaining services for cows detected in oestrus could be a major constraint in many regions of Sri Lanka. Obviously this clearly suggests the need of more intensified AI coverage with reduction in operational area of AI technicians coupled with enhanced mobility and communication.

Variations in fertility were also observed due to bulls. Of the seven bulls from which semen had been used for at least 20 inseminations, one had very poor CR (18.2%), while two others had CRs below 45%. Assuming that the other variables emanating from farm, technician and cow factors are randomly distributed across the sample of smallholdings, the finding of significant differences among bulls seem to suggest that the source of semen may of significant factor in the observed fertility differences among cows in the study. Thus it is conceivable that the continued use of such bulls in an AI programme is clearly unwarranted. The introduction of a regular programme, therefore is an urgent need to monitor each bull used in AI and to cull those that have low fertility. Semen produced locally gave higher CR than imported semen. Assuming that the imported semen was of good quality at the point of origin, problems during subsequent transport, storage and/or handling could have been responsible for the decline in fertility. Also, higher CR was obtained with chilled semen than with frozen semen. The former generally requires less stringent facilities and skills in storage and handling than the latter. This further stresses the need for special care in all operations associated with the use of frozen semen. The findings of this study emphasize the need for provision of optimal conditions for transport, storage and handling of semen, and also for routine monitoring of the quality at the point of receipt, during storage and prior to field use.

Factors related to the technique of performing AI are also very important determinants of CR in field AI programmes. These are influenced not only by the skill of the technicians, but also by their motivation, attitudes and the facilities available to them. In the present study only one technician was monitored in some of the locations and therefore location was a confounding factor in interpreting the influence of technicians on CR. However, the wide range of CR seen in individual technicians (32.3 - 58.9 %) is noteworthy. Further studies should therefore attempt to partition the effects of other factors such as location, bull and semen type in order to evaluate the true effects of the technician factor on CR. This would permit the identification of any needs for improving the facilities available to them, or for further training to improve their skills and attitudes.

CONCLUSION

The preliminary findings of this study highlight constraints which are most likely to negate the expected impact of AI on dairy cattle production. The major constraints to achieving high success rates from AI in the study area appear to be: poor heat detection by small-holder farmers; asynchrony between standing oestrus and AI; source, type and handling of semen in transport and storage and sub-optimal performance of AI technicians. Further, this study also highlights the value of RIA of milk progesterone as a routine diagnostic tool for monitoring the success rate and identifying the constraints for optimal utilization of AI, which can play an important role in increasing milk production in the wet zone areas of Sri Lanka.

ACKNOWLEDGMENTS

We thank the officers in the Division of Livestock Resources Development of the Department of Animal Production and Health, and the field veterinarians and AI technicians in Gampola, Kundasale, Teldeniya, Udunuwara and Yatinuwara VS ranges for their collaboration and assistance. We are also grateful to the farmers for their cooperation, and to Mr. A.M. Karunaratne, Mr. Janaka Herath, Ms. Ramani Ruwanpathirana and Dr. Ajith Dharmaratne for technical assistance.

REFERENCES

Abeygunawardena H and Amarasekera SKR 1995 Strategies for Revitalizing the Dairy Industry in Sri Lanka. Consultancy Report, Ministry of Livestock Development and Rural Industries, Colombo, Sri Lanka.

Abeygunawardena H, Mya Sein C and Epakanda LWB 1995a Status of the artificial insemination programme, success rate and factors affecting fertility of artificially bred cattle. Sri Lanka Vet. J. 42: 25-26.

Abeygunawardena IS, Abeygunawardena H, Perera ANF, Jayapadma HMHL and Epakanda LWB 1995b Effects of body condition, body weight and milk yield on postpartum ovarian function in cattle in mid country small holder production systems: A preliminary report. Sri Lanka Vet. J. 42: 31.

Abeygunawardena H, Abayawansa WD and Perera BMAO 1996 Comparative study of reproductive and productive characteristics of swamp and exotic river buffaloes in Sri Lanka. In: Role of Buffalo in Rural Development in Asia, Proceedings of SAREC/NARESA Regional Symposium, 11-15 December 1995, Peradeniya, Sri Lanka. pp. 321-336.

Alexander PABD, Abeygunawardena H, Perera BMAO and Abeygunawardena IS 1997

Current status and factors affecting the success of artificial insemination in smallholder farms in the mid country wet zone of Sri Lanka. Trop. Agric. Res. 9:204-216.

Dargie JD and Perera BMAO 1994 Training and transfer of technology related to immunoassay use in livestock production and health. World Anim. Rev. (FAO) 80-81: 46-52.

Garcia M, Jayasuriya MCN and Perera BMAO 1995 Improving animal productivity by nuclear techniques. In: IAEA Yearbook 1995. International Atomic Energy Agency, Vienna, Austria. pp: B17-B32.

Livestock Development Strategy 1988 Ministry of Rural Industrial Development, Colombo, Sri Lanka.

Mohamed AR, Sivakanesan R and Rajamahendran R 1990 Oestrous detection and reproductive performance of cattle in Sri Lanka. In: Studies on the Reproductive Efficiency of Cattle Using Radioimmunoassay Techniques. International Atomic Energy Agency, Vienna. pp. 73-85.

Mohan V, Kuruwita VY, Perera BMAO and Abeygunawardena H 1990. Effects of suckling on the resumption of post-partum ovarian activity in buffaloes. Trop. Agric. Res. 2:306-315.

Perera BMAO, Pathiraja N, Buvanendran V, Abeywardena SA and Piyasena RD 1978 Plasma progesterone levels during natural and prostaglandin synchronized oestrous cycles in buffaloes. Ceylon Vet. J. 26: 29-34.

Perera BMAO and Abeyratne AS 1979 The use of nuclear techniques in improving reproductive performance of farm animals. World Anim. Rev. (FAO) 32:2-8.

Perera BMAO, de Silva LNA and Karunaratne AM 1984 Studies on reproductive endocrinology and factors influencing fertility in dairy and draught buffaloes in Sri Lanka. In: The Use of Nuclear Techniques to Improve Domestic Buffalo Production in Asia. International Atomic Energy Agency, Vienna. pp: 13-28.

Perera BMAO, Pathiraja N, Abeywardena SA, Motha MXJ and Abeygunawardena H 1980 Early pregnancy diagnosis in buffaloes from plasma progesterone concentration. Vet. Rec. 106:104-106.

Perera BMAO 1986 Use of radioimmunoassay methods for studies on reproduction of buffaloes. In: Nuclear and Related Techniques in Animal Production and Health. International Atomic Energy Agency, Vienna. pp: 179-187.

Perera BMAO, de Silva LNA, Kuruwita VY, and Karunaratne AM 1987 Postpartum ovarian activity, uterine involution and fertility in indigenous buffaloes at selected village locations in Sri Lanka. Anim. Reprod. Sci. 14: 115-127.

Perera BMAO, Mohan V, Kuruwita VY and Abeygunawardena H 1992 Effects of suckling and national supplementation on postpartum ovarian activity and LH profiles in indigenous buffaloes in Sri Lanka. In: Proc. of the 12th International Congress on Animal Reproduction, The Hague, The Netherlands. Vol. 4:2081-2083.

Peters AR and Ball PJH 1995 Reproduction in cattle, Second Edition, Blackwell Press, Oxford, U.K.

Rajamahendran R and Thamotharam M 1983 Effect of progesterone releasing intravaginal device (PRID) on fertility in postpartum buffalo cow. Anim. Reprod. Sci. 6: 111-118.

Soni BK, Abeygunawardena H, Jayatilake MWAP and Kendaragama T 1991 Review of Animal Health and Extension Services of Department of Animal Production and Health in Sri Lanka. GTZ Consultancy Report, Ministry of Agricultural Development and Research, Peradeniya, Sri Lanka.

Sri Lanka Livestock Statistics 1991/92 Ministry of Agricultural Development and Research, Colombo, Sri Lanka.