J. Natn. Sci. Coun. Sri Lanka 1981 9(2) : 149—152

Comparison of Three Diluents for the Preservation of Buffalo Semen at Room Temperature

R. RAJAMAHENDRAN, M. THANGARAJAH AND P. THANGARAJAH
Department of Animal Husbandry, University of Peradeniya, Peradeniya, Sri Lanka.

(*Date of receipt* : 29 *September* 1980)
(*Date of acceptance* : 27 *April* 1981)

Abstract : Coconut milk extender (CME), Illini variable temperature (IVT) diluent, and Eggyolk citrate (EYC) were used to preserve buffalo semen at room temperature (26°-30°C). Comparison of the three diluents showed that IVT could maintain 50% sperm motility for 3.3 days and CME for 2.5 days, if semen had initial motility greater than 80%. Semen extended with EYC lost motility within 24 hours.

1. Introduction

Low temperature ranging from 5°C to 196°C have been used widely and successfully for the preservation of semen of both cattle and buffaloes. However, in the developing countries, where the buffalo is an important animal resource that must be improved, such low temperature preservation facilities are both costly and scarce. Hence the search for suitable diluents that could extend the life of buffalo semen stored at room temperature. Diluents that have been developed and proved satisfactory for preserving bull semen at room temperature have been tried with buffalo semen as well.[2] Illini variable temperature (IVT) diluent developed by VanDeMark and Bartlett and Cornell University Extender (CUE) developed by Foote and Bratten have been used with some success for the preservation of buffalo semen at room temperature.[4] Coconut Milk Extender (CME), Egg-Yolk Citrate (EYC) and Citric Acid Whey (CAW) have also been tried by different workers.[1,3,5,7] In our study, CME, IVT and EYC were evaluated for their ability to preserve buffalo semen at room temperature.

2. Experimental

This study was conducted during January to April 1979. Semen for preservation was obtained from 4 Murrah buffalo bulls roughly 4 to 5 years of age. The semen was collected from each bull twice a week at 8 a. m., by the artificial vagina method. Three false mounts were allowed before each collection. Immediately after collection, the semen was assessed for volume, motility, concentration, % dead sperms and % abnormal sperms. From each bull six ejaculates, each with motility greater than 60% were used for preservation. Each ejaculate was divided into three parts and each part was diluted with either CME or IVT or EYC. The

semen was extended with the above diluents to give a dilution rate of 1:20. Samples from extended semen were taken for examination of motility and live/dead sperm count.[6] Each part of the diluted semen was then subdivided into six, put into 2 ml glass vials and sealed with cotton plugs. The vials were then kept in a dark place with temperature ranging from 26°C to 30°C. At 24 hour intervals one vial from the stored samples were examined for motility and live/dead sperm count. The length of time to which each diluent could maintain sperm motility greater than 50% was noted. The composition of the diluents used in this study are given in Table 1.

TABLE 1. The composition of three diluents

1. Coconut Milk Extender — (CME)	Coconut Milk	—	100 ml
	Distilled water	—	100 ml
	Sodium citrate	—	4.32 g
	Calcium carbonate	—	100 mg
2. Illinivariable Temperature diluent (IVT)	Sodium citrate	—	20 g
	Sodium bicarbonate	—	2.1 g
	Potassium chloride	—	0.4 g
	Glucose	—	3.0 g
	Sulphanilamide	—	3.0 g
	Distilled water	—	1000 ml
Carbon dioxide was bubbled in until pH dropped to 6.30. Egg-yolk was then added at a rate of 10% by volume of the diluent			
3. Egg-Yolk citrate diluent	Distilled water	—	75 ml
	Egg-yolk	—	25 ml
	Sodium citrate	—	2.9 gm
To each diluent, 1000 IU of Penicillin and 1000 μg of Streptomycin were added per ml of diluent.			

3. Results and Discussion

The semen characteristics of freshly ejaculated semen of the 4 Murrah bulls used in this study are shown in Table 2. These values are in agreement with previous reports from India. The percentage motility during storage in the three diluents and percentage of dead sperms during storage are given in the Tables 3 and 4 respectively. Table 5 gives the number of days that each diluent could maintain 50% sperm motility. The results obtained in this study indicate that IVT diluent could maintain 50% sperm motility for 3.3 days and CME for 2.5 days, if semen had initial motility greater than 80%. Semen extended with EYC lost motility within 24 hours and therefore cannot be used for room temperature preservation of buffalo semen. Statistical analysis indicate that the difference in storage length between IVT and CME was not significant.

TABLE 2. Semen characteristics of Murrah Buffaloes

Bull No.	I	II	III	IV
Mean volume in ml	4	3.5	3.0	4.0
Mean Percent motility	82	76	71	84
Mean sperm concentration per ml	7.5×10^6	7.3×10^6	3.1×10^6	9.7×10^6
Mean percent dead sperms	7	8	10	6
Mean percent abnormal sperms	5	7	6	7

TABLE 3. Percent motility during storage at room temperature in the different diluents

Diluents	CME				IVT				EYC			
Bull Nos.	I	II	III	IV	I	II	III	IV	I	II	III	IV
Time (hrs)	85*	70	68	82	85	70	68	82	85	70	68	82
0												
24	70	58	55	70	72	60	55	72	70	12	—	18
48	55	50	38	60	60	52	42	60	—	—	—	—
72	50	40	24	52	54	41	28	55				
96	45	30	—	36	38	24	—	50				

* Average of six ejaculates

TABLE 4. Percentage dead sperms during storage at room temperature in the different diluents

Diluents	CME				IVT				EYC			
Bull Nos.	I	II	III	IV	I	II	III	IV	I	II	III	IV
Time (hrs)	7*	8	10	6	7	8	10	6	7	7	18	6
0												
24	14	20	22	12	11	12	21	12	48	42	60	48
48	28	31	38	22	20	24	40	31				
72	34	48	52	26	31	42	—	29				
96	45	—	—	38	38	—	—	36				

*Average of six ejaculates

TABLE 5. Storage length in days of buffalo semen upto 50% motility in CME, IVT and EYC diluent

Bull	CME	IVT	EYC
I	2.5 ± 1.58	3.33 ± 1.82	1
II	1.2 ± 0.98	1.8 ± 0.89	1
III	1.1 ± 1.08	1.6 ± 1.15	1
IV	2.6 ± 1.63	3.5 ± 1.87	1

Norman *et al*[5] preserved semen in CME at room temperature for 2 days, while Bhattacharya and Mathur[1] reported a possible storage length of 3 days. Our results of a storage length of 2.5 days was in close agreement with above findings. The IVT diluent used by us could maintain 50% motility for 3 to 3.5 days. However, Sengupta and Chaube[9] using modified carbonate diluent containing 0.1% cyctine hydrochloride and 4500 IU of catalase per 100 ml of semen and having pH of 6.1, reported that semen could be stored at room temperature for 144 hours if diluted at a rate of 1:25. EYC was found to be of no value in preserving buffalo semen at room temperature for periods longer than one day. No successful studies have been reported by other workers as well.[4] An interesting observation from our study was the apparent high correlation between initial motility and preservability, as can be seen when ejaculates of bulls I and IV and II and III are compared. Saxena et al[8] also reported that preservability or Murrah semen depends largely on initial motility.

Acknowledgements

The authors gratefully acknowledge the assistance given in the form of research grant by the Department of Animal Production and Health and the National Science Council of Sri Lanka.

References

1. Bhattacharya, M.K. & Mathur, A. L. (1978), *Indian Journal of Animal Sciences.* **48** (1) : 7
2. Dugwekar, Y. G. & Mittal, V. P. (1972), *Indian Veterinary Journal,* **49** : 156.
3. Ganguli, N. C., Bhosrekar, M. & Stephen, J. (1973) *J. Reprod. Fert.* **35** : 355.
4. Kaker, M. L. (1972) *Ceylon Vet. J.* **20** (4): 102.
5. Norman, C., Joshi, N. H., Abdullah Khan, C. K. & Rai, A. V. (1968) *Mysore, Journal of Agricultural Sciences.* **2**: 321.
6. Rajamahendran, R., Hayashi, T. & Dharmawardena, J. (1979). *J. Natn. Sci. Coun. Sri Lanka,* **7** (2) : 111.
7. Roy, A., Srivastara, R. K. & Pandey, M. D. (1955) *Curr. Sco.* **24**: 246.
8. Saxena, S. B., Tripathi, S. S. & Manglik, U. P. (1978) *Indian Journal of Animal Sciences.* **48** (9) : 648.
9. Sengupta, B. P. & Chaube, L. K. (1972) *Indian Journal of Animal Sciences.* **42** (3) : 163.