Semen
Repeat Breeder ✓
Anestrus

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EDITORIAL

We are happy to note that the Government of India is formulating a separate policy of Agriculture Development in the country.

The draft document on Agricultural Policy circulated among the Universities contains 31 paragraphs dealing with Agriculture, Animal Husbandry, Fisheries etc Out of this, only one paragraph (Para No. 10) deals with Animal Husbandry The attention paid to Animal Husbandry is superficial and did not deal with all aspects of livestock development in the country. The market value of Livestock population is about Rs. 45,000 crores. The contribution of Livestock by way of milk, work, wool. dung, meat, by products and eggs to the National income is to the tune of Rs. 45,000 crores per annum It is not out of place to mention that India with an annual milk production of 50 million tons ranks Third in the world after Soviet Union and America.

Milk and Milk products play a vital role in the country's agricultural economy, being the second largest contributor to the gross agricultural produce. The value of the milk and its products rank after rice, but before wheat. Hence milk can be regarded as India's second most important agricultural commodity. Dairying provides sustenance to millions of farmers in the country. In addition, production of draught bullocks is the most important function of Indian cattle.

Inspite of magnificient contribution of Animal Husbandry, this sector continues to receive cindrella treatment.

The time is now ripe for the formulation of a National Animal Science Policy to strengthen the National Economy.

– PRESIDENT ISSAR AND EDITORIAL BOARD

सदस्य योजना आयोग
योजना भवन नई दिल्ली–११०००१
Member Planning Commission
Yojana Bhavan New Delhi–110001

D. O. No. /PC/M(JP)/91
October 4, 1991

Dear Dr. Kaikini,

Many thanks for your letter of 27 th September, 1991 sending your good wishes on my appointment as Member, Planning Commission. With the good wishes of friends like you, I hope to be able to contribute to the planning process of the country.

I will read the IJAR Journals with great interest and hope they will be a source of inspiration for me in formulating the Eighth Five Year Plan.

With my very best wishes.

Dr. A. S. Kaikini
Editor,
Indian Journal of Animal Reproduction,
B–306 Ujwal Flats, Rahate Colony,
Jail Road, Nagpur–440 002

Yours sincerely,
(Sd/–)
(Jayant Patil)

First Announcement

NATIONAL SYMPOSIUM ON "RECENT ADVANCES IN CLINICAL REPRODUCTION IN DAIRY CATTLE" AND THE TENTH NATIONAL CONVENTION OF INDIAN SOCIETY FOR THE STUDY OF ANIMAL REPRODUCTION (ISSAR).

The Tamil Nadu chapter of the Indian Society for the study of Animal Reproduction (ISSAR) in association with Tamil Nadu Veterinary and Animal Sciences University and the Indian Council of Agricultural Research will be holding a National Symposium on "Recent Advances in Clinical Reproduction in Dairy Cattle" and the National Convention of ISSAR at Madras, tentatively from **19 th to 21 st March 1992.**

Scientist/Researcher interested to participate should send the abstracts of their scientific papers tentatively by 31-12-1991 and should submit full text of the paper in duplicate by 31-1-92. The delegate fee Rs. 300/– may be sent by Bank demand draft, drawn in favour of the Treasurer, ISSAR (Tamilnadu Chapter), Madras to the Organising Secretary before 10 th February 1992.

For further details, please contact :

Professor and Head,
Department of Obstetrics and Gynaecology
Madras Veterinary College, Madras-600 007
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Dr. J. RAJASEKARAN Ph. D.
The Organising Secretary
Tenth National Convention of ISSAR.

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ISSAR News

From Secretary's Desk

Book Review

C.R. SANE ORATION FUND
AN APPEAL

The Indian Society for the Study of Animal Reproduction (ISSAR) has instituted the "C.R. SANE ORATION" in honour of Dr. C.R. Sane, Founder President of ISSAR, for his yeoman service towards Animal Reproduction. He is responsible for guiding ISSAR since its inception in 1972 and has shaped the organisation into a valuable institution.

The first C.R. Sane Oration Lecture was delivered by the renowned Swedish Scientist, Dr. I. Settergren in December, 1986. The Second Oration was delivered by Prof. B.R. Deshpande in August, 1988 at the 7th Annual convention of ISSAR held at Trichur. To sustain this activity on a durable basis, ISSAR appeals for your generous contribution towards the "C.R. SANE ORATION FUND".

Your contribution may kindly be sent to the Treasurer, ISSAR, Dr. S.R. Pattabiraman, Professor of Clinics, Madras Veterinary College, Vepery, Madras — 600 007.

A. RAMAMOHANA RAO
PRESIDENT — ISSAR

IJAR:12:2:111 – 113 :1991

Evaluation Of Crossbred Bulls For Breeding Soundness

K. BABU RAO[1] and A.R. RAO[2]

Department of Animal Reproduction and Gynaecology, College of Veterinary Science, Tirupati-517502.

ABSTRACT

Out of 48 crossbred bulls evaluated, 23 (47.92%), 12 (25%) and 9 (18.75%) bulls were found to have satisfactory, questionable and unsatisfactory breeding soundness, respectively. Four (8.33%) crossbred bulls were unclassified as semen could not be collected from them.

* * *

Use of highly fertile bulls with superior genetic make up is essential for getting optimum results in A.I. programme. Indiscriminate use of breeding bulls without proper evaluation poses a potential threat to livestock industry as they may transmit undesirable genes or traits, besides contributing to poor fertility of the herd. Evaluation of bulls for breeding soundness is done by certain easily measurable criteria like scrotal circumference, sperm motility and sperm morphology (Simon, 1976). The present study was aimed to evaluate the crossbred bulls for breeding soundness.

Materials and Methods

A total of 48 crossbred bulls having 50% and 75% exotic inheritance, stationed at AICRP on Cattle, Lam Farm, Guntur, Andhra Pradesh, constituted the material for the study. History of each bull was collected from the records. A thorough clinical examination for general health was done and each bull was graded as good, fair or poor. Special attention was paid to detect structural and genital abnormalities and lesions, if any.

The evaluation of semen samples over a

Table-1 : Abnormalities of genital organs of crossbred bulls

S No.	Genital organ	Abnormality	Number of Bulls	Percentage
1.	Scrotum	Asymmetric	2	2.94
2.	Testis	1. Monorchid	6	8.32
		2. Asymmetric	2	2.94
		3. Held close to abdomen	1	1.47
		Total	9	12.73
3.	Epididymis	Unequal displacement of cauda	1	1.47
4.	Penis	1. Cork screw	2	2.94
		2. Large size penis	2	2.94
		Total	4	5.88

1. Assistant Research Officer, Livestock Research Station, Lam Farm, Guntur-522 034 (A.P.).

2. Dean of Post-Graduate Studies, A.P. Agricultural University, Rajendranagar, Hyderabad-500 030 (A.P.).

period of 6 months per bull was done as per Rao (1971). After evaluation of semen for individual motility, concentration, live sperm percentage and total abnormal spermatozoa, each characteristic was graded as very good, good, fair and poor as per Carroll *et al.*, (1963) and Zemjanis (1970), with modifications.

Finally, the bulls were classified as satisfactory, questionable and unsatisfactory when the total score was 60 and above, 30-60 and less than 30, respectively (Hill *et al.*, 1959 and Carroll *et al.*, 1963).

Results and Discussion

The general health was found to be good, fair and poor in 42 (87.50%), 4 (8.33%) and 2 (4.17%) crossbred bulls respectively. The incidence of abnormalities of scrotum, testis, epididymis, penis and hoof was noticed in 2.94, 12.73, 1.47, 5.88 and 10.29 per cent of crossbred bulls respectively (Table-1).

The semen quality was found to be satisfactory, questionable and unsatisfactory in 23 (47.92%),12 (12.0%) and 9 (18.75%) crossbred bulls respectively. Semen could not be collected from 4 crossbred bulls as they did not respond to A.V. The average motility (%), concentration (millions per ml.), live sperms (%) and total sperm abnormalities (%) in satisfactory bulls were found to be 82.09, 1220.83, 87.27 and 15.83 respectively, which were within the normal range reported for crossbred bulls (Rao and Rao, 1978; Garg and Pandit, 1983). In questionable and unsatisfactory bulls, the quality of semen was poor with low motility, poor concentration, lower livesperms, and high sperm abnormalities (Table-2).

The scrotal circumference (Table-2) was higher in "Satisfactory" bulls (34.56 cm) than in questionable (30.99 cm) and unsatisfactory bulls (27.75 cm), which is in agreement with the findings of Bangso *et al.*, (1981) and Bartlett, (1984).

The conception rate was higher in satisfactory bulls (50.58%) as compared to questionable (33.75%) and unsatisfactory (23.33%) bulls. The questionable and unsatisfactory bulls had higher percentage of sperm head abnormalities, loose heads, and knobbed acrosomal abnormalities, which might have adversely affected their fertility.

Out of 48 crossbred bulls evaluated, 23 (47.92%) bulls had satisfactory, 12 (25.0%) bulls had questionable and 9 (18.75%) bulls, had unsatisfactory breeding soundness and 4 (8.33%) bulls were unclassified as semen could not be collected from them. The present findings were allied to those reported by Deka *et al.*, (1982), but lower than that reported by Carroll *et al.*, (1963) and Bangso *et al.*,(1981).

It is evident from this study that a higher percentage of crossbred bulls had questionable and unsatisfactory breeding soundness with lower conception rates. It is therefore obvious that proper systematic evaluation and selection of bull is essential to improve the breeding efficiency in crossbred bulls.

Acknowledgements

The authors are grateful to the Senior Scientist (AB), Cattle Project, Lam Farm, Guntur for the facilities provided and to the Principal, College of Veterinary Science, Tirupati for the permission given to publish this paper.

Table 2 : Scrotal circumference and semen characteristics of crossbred bulls (Mean ±SE)

S. No.	Category of Bulls	No.of bulls	Scrotal circum ference (cm)	Motility (%)	Concent-tration of spermatozoa ($x10^6$/ml)	Live sperms (%)	Abnormalities of spermatozoa (%)					
							Proxi-mal drop-lets	Loose head	Head abnor mali ties	Acro-somal abnor-mali-ties	Mid piece abnor-mali-ties	Tail abnor-mali-ties
1	Satisfactory	23	34.56 ±2.85	82.09 ±2.15	1220.83 ±99.26	87.27 ±2.42	1.84 ±0.57	1.48 ±0.71	1.90 ±0.51	1.35 ±0.64	0.84 ±0.57	8.42 ±1.87
2	Questionable	12	30.99 ±2.29	69.13 ±4.04	938.13 ±205.21	75.81 ±4.73	4.68 ±1.79	4.80 ±3.26	3.47 ±1.57	4.15 ±2.78	1.60 ±0.69	10.70 ±2.10
3	Unsatisfac-tory	9	27.75 ±1.85	58.83 ±20.18	831.48 ±185.20	65.76 ±21.80	6.60 ±4.68	15.25 ±7.91	5.39 ±6.11	11.93 ±9.24	1.72 ±0.96	8.98 ±4.84

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Effect Of Induced Testicular Degeneration On Spermiogram In Bulls

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ABSTRACT

Two bulls were subjected to testicular degeneration through an insulation device for 120 hours. The semen of both the bulls was collected at weekly or bi-weekly intervals for 4 weeks before scrotal insulation (SI) and for 20 weeks after SI. Semen characteristics such as mass activity, motility, total number of sperm and abnormal spermatozoa showed considerable changes after SI and recovered only by twelve weeks of SI.

* * *

Testicular degeneration is one of the most common cause of reduced fertility or sterility in male domestic animals. Unlike any other organ in the body, the testes react rapidly and severely to all possible external influences by degeneration (Lagerlof, 1934). The varied nature of the possible causes of testicular degeneration makes the positive diagnosis very difficult, if not impossible. The incidence and general importance of bull infertility is not clearly determined though it is well known that economic losses do occur due to lowered fertility.

Materials and Methods

Two bulls, one Swedish Red and White (SRB) (Bull 'A') and one Swedish Friesian (SLB) (Bull 'B') were used in the study. They were 20 and 31 months old, respectively at the beginning of the experiment and were fed standard ration.

(A) *Semen collection and evaluation* : Four semen samples were collected at weekly intervals using artificial vagina to determine the spermiogram before SI. A total of 12 semen collections were made in 20 weeks after SI. The spermiogram included volume, density, mass activity, motility, total sperm count and sperm morphology. Sperm morphology was studied according to routine method reported by Rao (1971).

(B) *Scrotal Insulation* : A double walled plastic bag (filled with insulation material between two walls) was used to induce degeneration. The device was used for 120 hours (5 days) for scrotal insulation.

Results and Discussion

Sperm motility showed decline during the heat stress which lasted for 10 weeks. (Fig. 1) The total sperm number showed decline from week 1 to 8 of SI in both bulls (Fig. 2).In wet smear examination, protoplasmic droplets, loose heads and acrosome defects increased in first week of SI and from week four to week 12 after SI in case of Bull 'A' (Table 1). In case of Bull 'B' this increase was seen from week 5, 10 and 12. These defects could not be determined during weeks 2 and 3 after SI in Bull 'A' and

1. National Dairy Development Board, Anand-388 001.

2. Veterinary Department, Govt. of Botswana.

3. Director, Swedish International Programme on Animal Reproduction, Clinic Centre, Uppsala.

during weeks 2 to 4, 6 and 8 in case of Bull 'B' because of too few a sperm in the ejaculates.

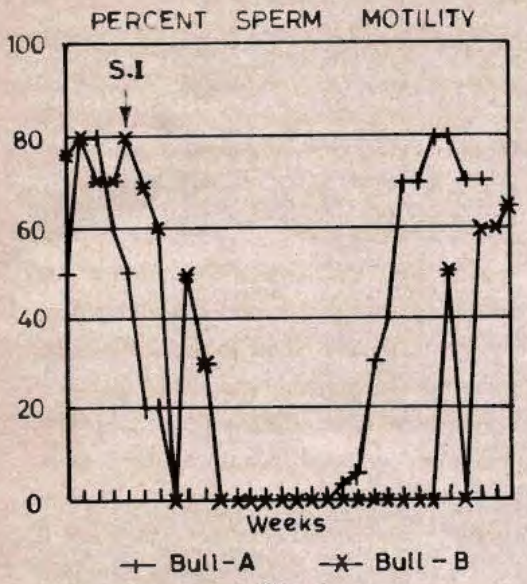

Fig. 1

Dry smear examination showed increase in abnormal sperm heads from week one of SI till week 10 in both bulls (Fig.3).

By week 12, spermiogram improved and was close to pre-SI levels in both bulls. Bull 'A' showed a tendency for an earlier regeneration. At 20 weeks, the spermiogram was again somewhat inferior.

It is well established that the testes of bulls must be at 4–5°C below body temperature for normal spermatogenesis. Elevation of intratesticular temperature by 4-5°C for only two hours will kill patchytene spermatocytes and B - spermatogonia (Amann, 1986).

The device used to induce testicular degeneration proved to be reliable in causing the desired effect. The ejaculate volume in both bulls seemed to be unaffected by the SI. This is is in agreement with Skinner and Louw (1966).

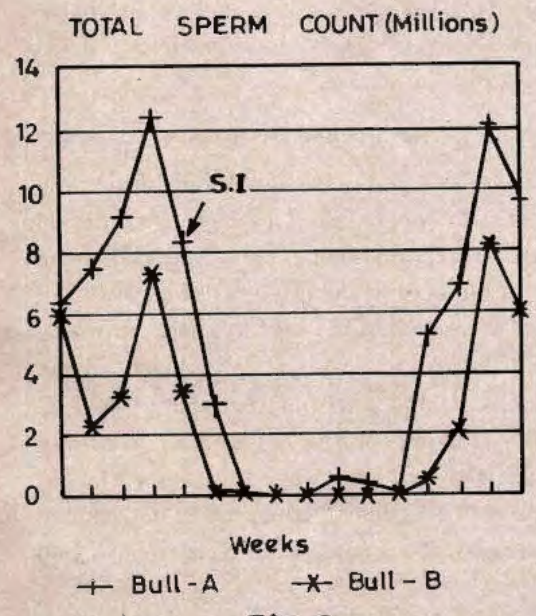

Fig. 2

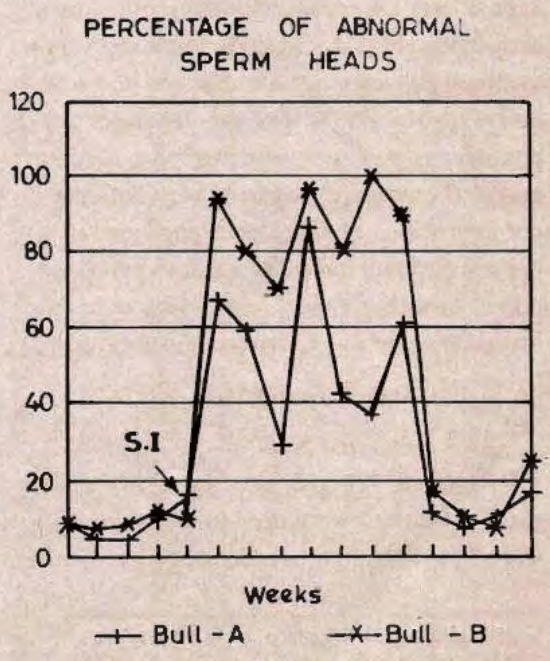

Fig. 3

The Sperm concentration decresed after one week of SI. This is in agreement with Gerona and Sikes (1970) and Ross and Entwistle (1979). The trend of increase in abnormal sperm numbers as well as changes in motility scoring was similar in both bulls.

The number of abnormal sperm heads increased in the first ejaculate after SI which is in agreement with the findings of Skinner and Louw (1966) and Gerona and Sikes (1970). The percentage of abnormal sperm heads was highest during week 1 to 8 of SI. The percentage of abnormal sperm was consistently higher in Bull 'B' than in bull 'A'.

Eleven weeks elapsed before regeneration, as indicated by the changes in sperm motility scoring, sperm concentration and percentage of abnormal sperm. Lagerlof (1934) carried out the pioneer classical experiments demonstrating that insulation of scrotum of bulls caused rapid testicular degeneration and recovery was a slow process. Dutt *et al* (1977) observed that when the temp. of testes is raised to 39°C in rams, it results in increased blood flow to testes. They also postulated that after five or six days of continuous exposure to high temperature, the blood flow decreased significantly due to higher levels of PGF_2 which causes constriction of spermatic artery in the pampiniform plexus.

The spermiogram at 20 weeks was inferior to that at 14 weeks indicating that bulls which have suffered from severe testicular degeneration may not recover completely within 12-14 weeks and repair of seminiferous epithelium is a very slow process. The study has confirmed that testicular degeneration causes changes in the spermiogram.

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Table 1 : Abnormal sperm % (Wet Smear)

		Week (– = Before scrotal Insulation)															
	Bull	–7	–6	–5	–4	SI	1	2	3	4	5	6	8	10	12	14	20
Prox	A	1.5	0.5	1.5	1.5	6.0	20.0	–	–	17.0	36.5	66.0	37.5	5.0	16.0	3.0	15.0
Drop-	B	5.0	2.0	2.0	1.5	6.0	0	–	–	–	12.0	–	–	30.0	7.0	3.0	8.5
Loose	A	1.5	0.5	1.5	1.5	8.0	55.0	–	–	39.0	9.0	3.0	4.0	10.0	0	1.5	5.0
Head.	B	5.0	2.0	2.0	1.5	15.0	0	–	–	–	1.0	–	–	2.5	0	2.0	1.0
Acro.	A	2.0	0.5	1.0	0	1.0	25.0	–	–	10.0	15.5	6.0	6.0	2.0	0	2.0	0.5
Abn.	B	1.5	2.0	0	0	14.0	0	–	–	–	3.5	–	–	1.5	0.5	0	0

0 = Nil, – = No sperm could be seen due to watery semen.

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Seasonal Variations In Relation To Freezability Of Semen Of Jersey And Crossbred Bulls With Varying Levels Of Exotic Inheritance.

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ABSTRACT

A total of 8,448 Semen ejaculates comprising 3,324 ejaculates of purebred Jersey (Jy), 336 of 75% Jy. crossbred, 1,903 of 62.5% Jy crossbred, 1,733 of 50% Jy.crossbred, 409 of 75% Holstein Friesian (HF) crossbred were studied for seasonal variations. The overall percentage of freezable ejaculates for Jy. purebred bulls, 75% Jy. crossbreds, 62.5% crossbreds, 50% Jy crossbreds, 75% HF crossbred bulls were 62.22%, 56.80%, 57.58%, 51.83% and 55.90% respectively. Seasonal and breed x seasonal effects were highly significant ($p < 0.1$) for each of the six genetic groups.

* * *

Due to the wide scale introduction of crossbreeding, a number of Frozen Semen Laboratories have been established throughout India. In cross-breeding Programme utilization of frozen semen of pure bred and crossbred bulls needs to be maximised. There is a great deal of breed and seasonal variation in initial and post-thaw motility. Hence it is necessary to study seasonal and breed x seasonal variations in respect of freezability to maximise utilization of frozen semen of bulls from different genetic groups. The present study was therefore undertaken.

Material and Methods

Studies were carried out during a period of

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4 1/2 Years (Nov.85 to Mar.90) on 31 bulls located at Frozen Semen Station, Nagpur. These comprised of six breed groups thus: 14 pure Jersey (Jy.); one 75% Jy; five 62.5% Jy.; seven 50% Jy; one 75% Holstein Friesian (HF) and three 62.5% H.F. crossbred bulls. Pure bred Jersey bulls ranged in age from 18 to 98 months, while cross bred bulls varied from 18 to 70 months of age. In crossbred bulls, the indigenous inheritance included various levels (12.5 to 50%) of Tharparkar, Gaolao, Hariana and Gir breeds. All the bulls were healthy, free from any infection or genital disorders and were maintained under identical conditions of feeding and management.

Semen ejaculates were collected by A.V. technique twice a week. Semen samples having density DD and above, mass activity + +(+) and above and initial motility of 70% and above were subjected to deep freezing. Tris egg yolk glycerol (6.%) dilutor was used. Antibiotics were added @ 1 mg/ml. dilutor. Four hours of equilibration time was given at 4° to 5°C. Horizontal vapour freezing technique (Land Shut Method) was used. Post-thaw motility (PTM) was observed 30 minutes after freezing as per Mathew (1974). Thawing was carried out in water at 57°C for 15 seconds. Frozen semen samples having 40% and above motility were considered freezable. Season and temp. range was as under:

S.No.	Season	Duration	Temp. range	Relative Humidity (RH)
1.	Winter	Oct. to Jan.	9° C to 30° C	40%
2.	Summer	Feb. to May	40°C to 45° C	29%
3.	Monsoon	June to Sept.	15°C to 35°C	55%

The relevant data was statistically analysed as per Snedecor and Cochran (1967).

Results and Discussions

(1) *Volume* :- The pooled over breed groups mean volume was 4.11± 0.02 ml. in monsoon, 3.62± 0.03 ml. in summer and 3.18 ± 0.02 ml. in winter season. Individual breed-wise means also indicate the maximum volume in monsoon followed by summer, with minimum volume recorded for all the breeds in winter. Kohli (1980) reported similar results in Jersey bulls. Porwal *et at* (1972) and Bhosrekar (1980) reported similar trend in case of Murrah buffalo bulls. In the present study HF crossbreds showed higher values for volume in rainy season (3.76 , 3.79 ml.), followed by summer (3.30 , 3.42 ml.) and lowest in winter (2.94 , 2.93 ml.) in case of 75% & 62.5% HF crossbred bulls respectively. However, Tomar *et al* (1985) reported higher values in summer (4.6 ± 0.2 ml.) followed by monsoon (3.91 ± 0.28 ml.) and lowest in winter (3.52 ± 0.23 ml) in case of HF x Hariana crossbred bulls. Analysis of variance for seasonal and breed x seasonal effects revealed highly significant ($P < 0.01$) influences. Critical difference test revealed maximum and similar mean volume in summer (3.46 ml.) and monsoon season (3.48 ml) with significantly less volume in winter (2.81 ml.) in Jersey pure bred bulls.

75% Jy. crossbred bulls however indicated maximum volume in summer (4.65 ml.) followed by monsoon (4.12 ml.) and least in winter (4.05 ml.). For other crossbred groups the volume was significantly highest in monsoon followed by summer and least in winter.

(2) *Mass Activity* :- The results revealed the pooled over breed groups mean mass activity of 2.81 ± 0.01 for summer season which was significantly superior (P < 0.01) to mean mass activity of winter and monsoon which was similar (2.68 ± 0.01 ml). Individual breedwise mass activity was best in summer, except in case of 62.5% HF crossbred bulls wherein mass activity was better in monsoon (2.72 ml.) than winter and summer with similar values (2.67 ml. and 2.65 ml.).

In case of Jersey pure breds, mass activity was best in summer (2.71) followed by monsoon (2.64) and lowest in winter (2.59). Kohli (1980) reported similar trend in case of 3 Jersey bulls in which higher values were reported in summer (4.26 ± 0.11) followed by monsoon (3.95 ± 0.13) and lowest in winter (3.68 ± 0.08) season. Bhosrekar (1980) reported best mass activity in monsoon (3.83), followed by summer (3.47) and lowest in winter (2.57) in murrah buffalo bulls, which is contrary to the findings in present study. In all other crossbreds, mass activity was best in summer followed by monsoon and lowest in winter, except in case of 62.5% HF crossbreds wherein mass activity was best in monsoon (2.72) and winter (2.67) and summer (2.65) values were similar. Tomar *et al* (1985) reported higher values in monsoon (2.43 ± 0.12) in case of HF x Hariana crossbred bulls.

Analysis of variance for mass activity revealed the highly significant (P < 0.01) influence of breed, season and breed x seasonal influences. Jersey purebred and crosbred bulls indicated superior mass activity in summer followed by monsoon and lowest in winter, except 62.5% HF crossbreds which showed significantly higher mass activity in monsoon than in summer and winter. It is evident that the mass activity of different genetic groups showed interaction to the seasons.

(3) *Initial Motility* :- Maximum values for pooled over breeds were recorded in winter (58.80 ± 0.34%), followed by summer (58.79 ± 0.47%) and lowest in monsoon season (50.16 ± 0.48%). However, individual genetic group showed better initial motility in summer followed by winter and lowest in monsoon season, except in case of 62.5% Jy. cross breds where winter values were best (59.16%) followed by summer (56.28%) and lowest in monsoon season (50.15%). Kohli (1980) also reported similar trend in case of three Jy pure breds, where initial motility was best in summer (85 ± 0.8%) followed by winter (83.7 ± 0.5%) and monsoon (83.1 ± 0.6%).

Analysis of variance for breed, season and breed x seasonal influences indicated highly significant effects (P < 0.01). One notable common feature for all breed groups is the significantly lowest initial motility in rainy season over the other two seasons.

(4) *Post Thaw Motility* (PTM):- The overall pooled over mean values of PTM were best in summer (45.03 ± 0.17%), with similar values in winter (43.0 ± 0.3%) and monsoon season (43.0 ± 0.35%). Purebred Jy bulls recorded better PTM in winter (43.65%) followed by summer (43.45%) and lowest in monsoon (40.35%). 75% Jy crossbred bulls however showed better PTM in summer (48.72%), followed by winter (43.50%) and lowest in monsoon (42.82%). 75% HF crossbred bulls also showed higher PTM in summer (39.63%), followed by monsoon (37.16%) and lowest in winter (33.15%). Analysis of variance indicated highly significant (P < 0.01) effect.

In the present study, Jy pure breds, 75% and 62.5% Jy crossbreds and 75% HF

Table 1 : Breed and Season-wise means of semen attributes of Jersey and Cross-bred bulls.

S. No	Parameters	BREED/GENETIC GROUP						Over all Pooled Over
		Jersey (Jy.)	75% Jy.	62.5% Jy.	50% Jy.	75% H.F.	62.5% H.F.	
1	**Volume (mL)**							
	Winter	2.81^{b}	4.05^{a}	3.51^{a}	3.48^{a}	2.94^{a}	2.93^{a}	$3.18^{a} \pm 0.02$
	Summer	3.46^{a}	4.65^{c}	3.70^{b}	3.92^{b}	3.30^{b}	3.42^{b}	$3.62^{b} \pm 0.03$
	Monsoon	3.48^{a}	4.12^{b}	3.95^{c}	5.78^{c}	3.76^{c}	3.79^{c}	$4.11^{c} \pm 0.02$
2	**Mass Activity (0-5 Scale)**							
	Winter	2.59^{a}	2.35^{a}	2.66^{a}	2.77^{a}	2.22^{a}	2.67^{a}	$2.68^{a} \pm 0.01$
	Summer	2.71^{b}	2.53^{b}	3.14^{b}	2.82^{b}	2.51^{b}	2.65^{b}	$2.81^{b} \pm 0.01$
	Monsoon	2.64^{c}	2.50^{c}	2.90^{c}	2.60^{c}	2.44^{c}	2.72^{c}	$2.68^{a} \pm 0.01$
3	**Initial Motility (%)**							
	Winter	60.95^{a}	51.59^{a}	59.16^{a}	58.81^{a}	57.20^{a}	56.61^{a}	$58.80^{a} \pm 0.34$
	Summer	61.11^{a}	65.70^{b}	56.28^{b}	60.80^{b}	58.53^{b}	59.69^{b}	$58.79^{a} \pm 0.47$
	Monsoon	51.48^{b}	41.41^{c}	50.15^{c}	54.13^{c}	41.27^{c}	51.16^{c}	$50.16^{b} \pm 0.48$
4	**Post-Thaw Motility (%)**							
	Winter	43.65^{a}	43.50^{a}	41.98^{a}	44.92^{a}	33.13^{a}	43.00^{a}	$43.00^{a} \pm 0.30$
	Summer	43.45^{a}	48.72^{b}	45.06^{b}	49.55^{b}	39.63^{b}	44.23^{b}	$45.03^{b} \pm 0.17$
	Monsoon	40.35^{b}	42.82^{a}	43.71^{c}	44.03^{c}	37.16^{c}	54.40^{c}	$43.00^{a} \pm 0.35$

Means bearing same superscripts do not significantly differ from one another.

Table 2 : ANOVA for Breed, Seasons and Breed × Season effects on Semen attributes of different breed groups.

S. No	Seminal attribute	SOURCES OF VARIATION			
		Breeds	Seasons	Breed × Season	Error
1	**Volume** df	5	2	10	8430
	Mean Squares	294.43^{xx}	618.13^{xx}	362.26^{xx}	1.98
2	**Mass Activity** df	5	2	10	8282
	Mean Squares	28.14^{xx}	23.98^{xx}	428.77^{xx}	0.42
3	**Initial Motility** df	5	2	10	7863
	Mean Squares	12138.24^{xx}	74669.76^{xx}	37904.73^{xx}	402.62
4	**Post-Thaw Motility** df	5	2	10	4859
	Mean Squares	4405.96^{xx}	551030.23^{xx}	4467.25^{xx}	492.57

xx denotes $P = < 0.01$

crossbred bulls have shown significantly superior response to volume, mass activity and initial motility and post-thaw motility in summer and monsoon season, whereas 50% Jy crossbred bulls showed better response in summer and winter, while 62.5% HF crossbred bulls showed better response in monsoon followed by similar response in summer and winter season. Bhosrekar (1990) also reported that exotic breeds show influence of season on donation and quality of semen. He also concluded that semen parameters in crossbred bulls were significantly affected by season.

In hot climate if bulls are protected against climatic odds, seasonal variation in summer production can be easily controlled (Bhosrekar, 1990).

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Studies On Chemical Composition Of Seminal Plasma Of Red Kandhari And Cross-bred Bulls

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ABSTRACT

Estimated levels of sodium, potassium, chloride, calcium and inorganic phosphorus were 71.06 m Eq.per litre, 16.18 m Eq.per litre, 149.80 mg.%, 14.97 mg.% and 4.03 mg.% in seminal plasma of Red Kandhari bulls and 67.23 m Eq.per litre, 27.60 m Eq.per litre, 117.65 mg.%, 20.93 mg.% and 3.27 mg.% in cross-bred bulls respectively. Significant difference only in potassium and calcium levels

of seminal plasma between Red Kandhari and Cross-bred bulls was observed

* * *

Red Kandhari is a recent ICAR recognised breed of cattle from Marathwada region. The present studies were undertaken to estimate the biochemical norms of Red Kandhari and Cross-bred bull seminal plasma.

Material and Methods

Two Red Kandhari and two Red Kandhari × Jersey (50 %) bulls were selected for the present study from the available lot of 'Cattle unit' of Marathawada Agricultural University, Parbhani. Semen samples were collected by A.V. method. Individual semen samples with optimum quality alone were used. The seminal plasma was collected by centrifuging the semen samples immediately after collection.

Seminal plasma samples were analysed for sodium, potassium, chloride, calcium and inorganic phosphorus levels. The estimation of sodium and potassium content was done by 'Flame-photometry' technique (Wotton, 1974), calcium as per Trinder (1960), chloride as per Schales and Schales (1941) and inorganic phosphorus as per Gomorri (1942). The data were analysed by Anova as per Snedecor and Cochran (1967).

Results and Discussion

Details of the estimated values obtained are presented in Table 1.

As per Roberts (1971) and Sane *et al.* (1982), sodium level ranges from 140-280 mg.% in semen and 152-370 mg.% in seminal plasma of bulls. Present findings are in agreement with their observations. However, Roberts (1971) has cited higher range of potassium (80-210 mg.%) of bull semen. Values of potassium level obtained in present studies are in consonance with that of Sane *et al.*, (1982).

The present findings of chloride level is within the normal range (110-293 mg.%) of seminal plasma reported by Sane *et al.* (1982) but are lower than that (247.70 mg) reported by Maule (1985).

Sane *et al.*, (1982) and Maule (1985) have reported higher Calcium levels in seminal plasma (37 mg %)and (34mg.%).

Inorganic Phosphorus level recorded is higher than the reports of Kulkarni (1973) in Tharparkar bulls, Sane *et al* (1982) and Maule (1985).

Statistical significant difference was found in the seminal plasma levels of potassium and calcium between Red Kandhari and cross-bred bulls. However, the difference in other values was not significant (Table 2).

Table 1: Estimated values of Biochemical constituents of Red Kandhari and Red Kandhari x Jersey (50 per cent) bull seminal plasma.

Sr.No.	Biochemical constituent	Red Kandhari pure-bred Mean values	Red Kandhari Cross-bred Mean values
1	Sodium (mEq. per lit.)	71.06	67.23
2	Potassium (mEq. per lit.)	16.18	27.60
3	Chloride (mg. %)	149.80	117.65
4	Calcium (mg. %)	14.97	20.93
5	Inorganic Phosphorus (mg. %)	4.03	3.27

Table 2: ANOVA of individual and breedwise variation of Biochemical constituents of Red Kandhari and Red Kandhari x Jersey (50 per cent) bull seminal plasma

Sources of Variation	Sodium		Potassium		Chloride		Calcium		Inorganic phosphorus	
	DF	MS	DF	MS	DF	MS	DF	MS	DF	MS
Between individuals	3	163.69	3	308.5	3	5217.01	3	80.99	3	6.64
Within individuals	19	50.31	19	97.24	22	2094.43	22	41.19	20	5.39
Between individuals	1	76.50	1	679.54*	1	6082.41	1	196.60*	1	2.83
Within individuals	21	65.26	21	99.69	24	2323.29	24	39.68	22	5.68

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Studies On Deep Freezing Of Buffalo Semen

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ABSTRACT

The semen of five Murrah buffalo bulls was frozen in four different diluters using different methods of glycerolisation, various periods of equilibration and different timings of freezing. The results indicated that Tris egg yolk diluter was suitable, when two step glycerolisation (6.8%) and 4-5 hours of equilibration (5° C) with ultra fast freezing (3 mts.) is used for deep freezing of buffalo semen.

* * *

Artificial Insemination in buffaloes has met with limited success. This is merely because of poor preservation quality of buffalo semen. The seasonal fluctuation in the quality of buffalo semen has posed many problems in the A.I. programme using chilled semen. The present studies were initiated to study the freezability of buffalo spermatozoa at ultra low temperatures.

Materials and Methods

Five Murrah buffalo bulls of about 4-5 years of age belonging to Indo-Swiss Project,

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Mattupatty, were included in the study. The semen was colleeted using A.V. and evaluated for its quality.

The study was divided into 4 experiments. In the first experiment, 4 semen diluters : CAW (Ganguli *et al* 1973), Egg yolk citrate (Salisbury *et al* 1941), Lactose egg yolk (Md. Shafi Ch., 1979), and Tris egg yolk (Steinbach and Foote, 1967) were evaluated using medium french straws and frozen in liquid nitrogen. In the second experiment, the selected diluter of experiment No. 1, was subjected to 4 different ways of glycerolisation thus : (i) The part A diluter contained 6% glycerol and part B, 6.8%. The part B was mixed with part A in two steps at an interval of 10 minutes, (ii) Part A of the diluctor was without glycerol and part B contained 12.8% glycerol. The part B diluter was added to part A in single step, (iii) The part A & B fractions of diluters were as in 2 but the part B was added to part A in two steps at interval of 10 minutes, (iv) The part A & B of the diluter were as in (ii), B part of the diluter was added to part A in 4 steps at an interval of 10 minutes.

In the 3rd experiment, the diluted semen samples in medium french straws were equilibrated (5° C) for time intervals ranging from

Table 1 : Pre-freezing and post thaw motility in different diluters with 6.8% glycerol.

S.No.	Diluter used	Mass Activity (0–3)	Initial motility (%)	Prefreezing motility (% ± S.E.)	Post thaw motility (% ± S.E.)
1	C A W	2.50	68.00	28.61 ± 4.82	7.24 ± 0.79
2	Egg yolk citrate	2.50	73.00	55.99 ± 2.31	16.86 ± 2.42
3	Lactose egg yolk	2.50	73.00	61.11 ± 1.22	24.74 ± 2.10
4	Tris egg yolk	2.50	75.00	66.10 ± 0.55	38.60 ± 1.34

Table 2 : Pre-freezing and post thaw motility after glycerolisation in various ways.

Method of glycerolisation	Mass activity (0 – 3)	Initial motility (Percent)	Pre-freezing motility (% ± S.E.)	Post thaw motility (% ± S.E.)
Glycerol in A diluter 6% and 6.8% in B; two steps of glycerolisation	2.50	73.00	68.91 ± 3.15	37.40 ±3.87
Glycerol 12.8% in B diluter, glycerolisation in single step	2.50	72.50	66.85 ±2.04	32.22 ±4.63
Glycerol 12.8% in B, diluter, glycerolisation in two steps	2.50	73.00	67.80 ±2.43	31.99 ±3.23
Glycerol 12.8% in B diluter, glycerolisation in four steps.	2.50	73.50	67.80 ±2.80	35.50 ±2.90

Table 3 : Pre-freezing and post thaw motility after equilibration at 5° temp.

Equlibration time (Hrs)	Mass activity (0 – 3)	Initial motility (%)	Pre-freezing motility (% ± S.E.)	Post thaw motility (% ± S.E.)
0	2.50	78.00	76.38 ±0.83	27.22 ±3.22
1	2.50	78.00	75.83 ±0.95	39.85 ±2.35
2	2.50	78.00	76.80 ±0.98	41.66 ±2.85
3	2.50	78.00	76.24 ±0.38	42.77 ±2.52
4	2.50	78.00	77.07 ±0.38	45.41 ±1.58
5	2.50	78.00	77.22 ±0.39	43.74 ±1.99
6	2.50	78.00	76.94 ±0.39	43.74 ±1.99

Table 4 : Pre-freezing and post thaw motility with different rates of freezing after adding selected level of glycerol and keeping it at best equilbration period.

Rates of freezing	Mass activity (0 – 3)	Initial motility (%)	Pre-freezing motility (%)	Post thaw motility (% ± S.E.)
Slow freezing (10 mts.)	2.50	78.50	76.00	41.49 ± 1.60
Medium freezing (7 mts.)	2.50	78.50	76.00	40.00 ± 0.39
Fast freezing (5 mts.)	2.50	78.50	76.00	39.66 ± 1.07
Ultra fast freezing (3 mts)	2.50	78.50	76.00	44.33 ± 0.85

0 to 6 hours. The diluter used was as selected in experiment No. 1 while glycerolisation technique was selected from experiment No. 2.

In the 4th experiment, the suitable diluent of experiment No. 1, the suitable glycerolisation procedure of experiment No. 2 and the suitable equilibration time of experiment No. 3 was used and the semen was frozen in LN_2 vapours with 4 different freezing rates: slow freezing (10 mts); medium freezing (7 mts); fast freezing (5 mts) and ultra fast freezing (3 mts).

Results and Discussion

The prefreezing and post-tbaw motility (PTM) of spermatozoa was seen under phase contrast microscope.

Maximum PTM was observed in Triseggyolk diluter (Table 1) and confirms the reports of Sharma *et al.* (1979), Vasanth (1979) and Singh Sall *et al.* (1980).

Treatment 1 gave maximum PTM (Table 2) However, analysis of variance revealed no significant difference between treatments.

4 to 5 hours of equilibration time resulted in maximum PTM (Table 3). These results are in accordance with the reports of Vasanth (1979), Md. Shafi Ch. (1979) and Singh Sall *et al.* (1980).

Highest mean PTM was obtained when ultra fast freezing was done (Table 4). Roy and Ansari (1973) reported better post thaw motility when slow freezing was practised.

From these studies it is concluded that Tris egg yolk diluter is suitable for deep freezing of buffalo semen and glycerolisation in two steps using 6.4% glycerol, 4-5 hours equilibration time and ultra-fast freezing gives maximum post thaw motility.

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Studies On Buffalo Sperm Morphology During Various Stages Of Freezing In Certain Extenders

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ABSTRACT

Efficacy of egg yolk citrate glycerol (EYCG), tris yolk glycerol (TYG) and citric acid whey glycerol (CAWG) extenders was studied at various stages (fresh semen, after complete extension, after equilibration period of 4 hours, 24 hours after freezing and 7 days after freezing) of buffalo bull semen freezing. A total of 40 ejaculates from five fertile bulls (eight from each) exhibiting more than 70% initial motility were evaluated for spem head abnormalities, sperm tail abnormalities and abnormal acrosomes. The head and tail abnormalities were higher in CAWG followed by EYCG and TYG at various stages of deepfreezing. TYG provided maximum protective action to the sperm acrosomes, followed by EYCG and CAWG. The most critical stage at which the sperm morphology was highly affected, was between equilibration period and 24 hours after freezing.

* * *

The relationship between the sperm morphology and fertility of semen is well documented. The methods of buffalo semen processing and preservation may bring about some alterations in the structure of spermatozoa, resulting in poor motility, livability and fertility. Kakar and Anand (1984), Rao *et al* (1986) and Krishna and Rao (1987) recorded increased percent acrosomal defects after freezing of buffalo semen. The present work deals with certain morphological changes in the spermatozoa during various phases of freezing of buffalo semen.

Materials and Methods

Forty ejaculates from five mature buffalo bulls (eight from each bull) were collected by A.V. (42-47°C) at weekly intervals. After recording physical characteristics, mass activity and individual motility, each ejaculate was evaluated for sperm head abnormalities (Lagerlof, 1936), acrosomal morphology and sperm tail abnormalities (Hancook, 1952) and then subjected to deep- freezing.

Three extenders - Egg yolk citrate glycerol (EYCG), tris yolk glycerol (TYG) and Citric acid whey glycerol (CAWG) were used. Their composition was as per Salisbury *et al.* (1941), Davis *et al.* (1963) and Ganguli *et al.* (1973), respectively. Penicillin-G-sodium (100 I.U./ml) and streptomycin sulphate (1 mg/ml) were added to all the extenders and their pH adjusted to 6.8. The final concentration of glycerol was 7% by volume.

Each ejaculate was divided into three fractions, which were diluted in the three extenders separately. Semen was frozen as per the technique of Cassou (1976) with four hours equi-

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libration period. Sperm abnormalities were studied in pre-freezing stages : stage 1 (fresh semen), stage 2 (after complete extension), stage 3 (after equilibration period) and post-freezing (PF) stages ; stage 4 (24 hours PF) and stage 5 (7 days PF). The data was analysed by applying F test (Snedecor and Cochran, 1967).

Results and Discussion

Head abnormalities : Significantly ($P < 0.01$) lesser head abnormalities were observed in TYG extended semen than in EYCG and CAWG extended semen at different stages of processing and freezing (Table 1). This could be attributed to differences in permeability of plasma membrane and individual resistance to changes in osmotic pressure (Lovelock, 1953; Farrant, 1965).

Increase in head abnormalities was significant ($P < 0.01$) between the stages of observations in the three extenders studied. The significant increase may be due to inclusion of acrosome while studying the sperm head morphology following William's stain technique, since about 60% of anterior portion was covered with acrosome (Salisbury *et al.*, 1985).

Although statistically significant ($P < 0.01$) increase in head abnormalities was meagre from neat semen to the stages of complete extension and equilibration, while higher proportions of head abnormalities were encountered following freezing and thawing. The present observations gain support from the report that dilution did not seem to have any apparent effect on spermatozoan structure (Saacke and Marshall, 1968) while protusion of anterior cap of spermatozoa due to vacuole formation or ablation was noticed following freezing and thawing (Healey, 1969 and Lung and Bahr, 1972).

Tail abnormalities : Tail abnormalities % did not differ significantly between the extenders at various stages of processing and freezing of semen (Table 1). Similar observations were made by Hundal (1987).

Incidence of abnormal sperm tail increased significantly ($P < 0.01$) from fresh semen to complete extension in all the three extenders, which could be attributed to effect of cooling (Salisbury *et al.*, 1985). Mann (1964) also observed that flagellum of mammalian cells could bend while cooling fresh semen to +5° C. Significant increase ($P < 0.01$) in tail abnormalities between equilibration and 24 hours of freezing could be attributed to the exposure of sperms to changed concentration of electrolytes and low temperature which could lead to tail abnormalities (Lovelock and Polge, 1954).

Intact acrosomes : Significantly ($P < 0.01$) higher intact acrosomes in TYG than in EYCG observed at all stages of processing and deep-freezing, could be attributed to better protective action of TYG.

Significant ($P < 0.01$) decrease in intact acrosome from stage 1 to stage 2, stage 3 to stage 4, and stage 4 to stage 5, could be due to labile nature of sperm acrosomal cap to chemical or physical processes involved (Bishop and Austin, 1957), alteration of sperm acrosomal cap following death of sperm cells accompanying the process of freezing (Hancook, 1953) and changes in acrosome accompanied with ageing of spermatozoa (Saacke and White, 1972). Similar observations were found by Hundel (1987).

Table 1 : Morphology of spermatozoa (%) in neat and extended buffalo semen during various stages of deep-freezing.

S. No.	Extender Studied	Parameters Studied		
		Sperm head abnormalities*	Sperm tail abnormalities**	Acrosomal abnormalities***
1.	**Fresh Semen**	2.03 ± 0.15	8.23 ± 1.05	4.47 ± 0.66
2.	**Egg yolk citrate glycerol (EYCG)**			
	(i) After complete extension	2.40 ± 0.08	22.51 ± 0.89	19.72 ± 2.41
	(ii) After equilibration period	2.80 ± 0.07	25.62 ± 0.84	24.35 ± 1.53
	(iii) 24 hrs post freezing	4.45 ± 0.14	30.63 ± 0.86	48.22 ± 1.95
	(iv) 7 days post freezing	4.76 ± 0.13	33.88 ± 0.96	58.03 ± 2.54
3.	**Tris yolk glycerol (TYG)**			
	(i) After complete extension	2.74 ± 0.08	21.75 ± 0.64	12.04 ± 1.22
	(ii) After equilibration period	3.12 ± 0.10	27.23 ± 2.07	16.84 ± 1.43
	(iii) 24 hrs. post-freezing	4.52 ± 0.18	32.40 ± 2.13	38.52 ± 1.44
	(iv) 7 days post-freezing	5.34 ± 0.11	34.80 ± 1.82	46.13 ± 1.22
4.	**Citric acid whey glycerol (CAWG)**			
	(i) After complete extension	2.46 ± 0.09	21.35 ± 1.75	13.50 ± 0.38
	(ii) After equilibration period	2.73 ± 0.08	24.26 ± 1.94	18.42 ± 0.41
	(iii) 24 hrs. post-freezing	3.98 ± 0.11	31.48 ± 1.63	45.55 ± 1.88
	(iv) 7 days post-freezing	4.25 ± 0.10	34.99 ± 0.96	54.02 ± 2.29

* CD = 0.17 ($P < 0.01$)
** CD = 5.40 ($P < 0.01$)
*** CD = 5.32 ($P < 0.01$)
= 3.99 ($P < 0.05$)

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Effect Of Pre-freeze Holding Time (at 5°C) And Thawing Rates On Post-Thaw Motility And Thermoresistance of Bubaline And Taurine Spermatozoa.

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ABSTRACT

Thirty six semen ejaculates, 6 from each of 3 HF and 3 Murrah bulls, were extended in Tris-fructose-egg yolk (10% for cattle; 5% for buffalo) - Glycerol extender keeping 18-20 million sperm per 0.5 ml French straw. The comparative response of two species to different prefreeze holding/equilibration times (0 and 2 hr at 5° C) and thawing rates (40°C/60s; 60° C/15s and 80°C/5s) on post-thaw motility (PTM) and thermoresistance (TR) of spermatozoa, was studied. Mean values for initial and pre-freeze motility were significantly ($P < 0.05$) higher in cattle (81..67 ± 1.28 and 72.78 ± 1.47%) than in buffalo (76.94 ± 1.81% and 67.76 ± 2.03%) semen. PTM did not vary between them (45.23 ± 1.71 vs 44.49 ± 1.99%). The post-thaw incubation/thermoresistance test showed significant deterioration in the motility of buffalo sperm than the cattle sperm (10.71 ± 1.42 vs 23.56 ± 1.77%). The individual bulls within the breed/species also differed significantly ($P < 0.01$) in their sperm motility at different steps of semen processing. A holding time of 2 hr at 5°C significantly ($P < 0.05$) improved the PTM (48.58 ± 2.29 vs 41.82 ± 2.10%) and TR (26.29 ± 2.55 vs 20.83 ± 2.21%) of cattle semen as compared to that of O hr. This effect, however, was not significant in buffalo sperms (46.02 ± 2.57 vs 42.96 ± 2.60 and 10.46 ± 1.88 vs 10.95 ± 2.38% for the two traits, resp.). Similarly, PTM increased significantly ($P < 0.01$) with increase in thawing temperatures from 40° to 80° C in cattle (40.56 ± 2.68

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to 49.17 ± 2.82%) and buffalo (38.47 ± 3.26 to 50.56 ± 2.91%) semen. But, the post-thaw thermoresistance values increased significantly only in cattle (18.33 ± 3.07 to 27.08 ± 2.49%) and not in buffalo semen (9.99 ± 2.56 to 11.66± 2.89%). A holding time of 2 hr over O hr at 5° C was advantageous for deep freezing, particularly of cattle semen, as well as the thawing rate of 80° C/5s or 60° C/15s over 40° C/60s for better post-thaw results in both the species.

* * *

There is a need to evolve an economic, simple, rapid and practical method of semen freezing and handling that would give acceptable fertility rates. Numerous investigators have shown beneficial effect on freezability, post-thaw longevity and fertility of frozen semen with reduced equilibration time and faster thawing rates in the bovines (Jondet *et at*, 1972; Robbins *et al.* 1972; Wiggin and Almquist, 1975; Aguirre *et al.*, 1978; Bhosrekar *et al.* 1984; Tuli *et al* 1985; Rao *et al.* 1986; Motwani *et al*, 1986; Sahni and Mohan, 1988, Muralinath *et al.*, 1990). Yet the optimum consistant time for equilibration and/or thawing semen has not been established. Therefore, an attempt was made to compare the effect of these two factors on post thaw quality of bull and buffalo bull semen.

Materials and Methods

Six semen ejaculates obtained from each of 3 Murrah and 3 HF bulls at weekly interval using A.V. were studied at the IVRI Germ Plasm Centre. Following routine evaluation, the semen samples were diluted at 32°C in tris-fructose-egg yolk (10% cattle; 5% buffalo) - glycerol (64%) diluent keeping 35-40 million sperm/ml and filled in 0.5 ml medium French straws. The semen straws were then placed in a bread box half filled with water (1 litre, 25-28° C), and cooled to 5° C in 2 hr by placing it in the freezing chamber of a refrigerator. Following cooling to 5° C (0 hr equilibration), three straws were frozen in liquid nitrogen vapour for 15 min. in a "Kool pack" (Sahni and Mohan, 1988). Rest of the straws were held/ equilibrated at 5° for 2 hr before deep freezing. The thawing of each lot was done in water bath at 40°C, 60°C and 80° C for 60, 15 and 5 seconds, respectively, after 24hr of storage in LN_2, the percentages of prefreeze (PFM) and post-thaw motility (PTM) were assessed under a phase contrast microscope (40 x) fitted with a biotherm (38°C). The thawed straws were then immediately transferred to an incubator at 38° C and reassessed for motility after 1hr of thermal stress. The data were statistically analysed in factorial RBD on a micro-32 computer taking the bull as random variable (Snedecor and Cochran, 1971).

Results And Discussion

Influence of species/bulls : The mean values of mass activity (3.28 ± 0.18 vs 2.25 ± 0.26) and the percentages of initial (81.67 vs 76.94%), prefreeze (72.78 vs 67.76%) and post thaw incubation (23.56 vs 10.71%) motility were significantly ($P < 0.05$) higher in HF bulls than in the Murrah buffalo bulls (Table 1) However, the motility of immediate post-thawing was almost identical between the two species (45.23 vs 44.49%). The individual bulls within the species also differed significantly ($P < 0.01$) in their sperm motility at all steps of semen processing, pre and post-freezing (Tables 1 & 3). The significant variations observed in the initial and post thaw quality of semen between bulls/breeds corroborated well with the earlier reports of Pavithran *et. al.* (1972), El-kafrawi and Barada (1974), Crabo *et. al.* (1980), Jani

(1982), and Bhavsar *et al.* (1989). Further the significantly poor post thaw thermoresistance of buffalo spermatozoa as compared to cattle sperm recorded in the present study also finds support from the reports of Motwani *et at.* (1986), Sahni and Mohan (1988a) and Muralinath *et al* (1990). However, Tuli *et al.* (1985), Rao *et al.* (1986) and Sahni and Mohan (1988[a]) reported fairly good post-thaw incubation survival for more than 3 hr in cattle as well as buffalo semen. This discrepancy may be due to the inherent quality of cattle and buffalo sperm, the composition of diluent (egg yolk, sugars & glycerol levels), sperm concentration, cooling time, equilibration, and freezing thawing procedures employed iu different studies.

Influence of equilibration time : The PTM and incubation survival of cattle semen was found to improve significantly ($P < 0.01$) following 2 hr of holding/equilibration period at 5°C before deep freezing, as compared to that frozen without any equilibration time. However, such effect was not observed in buffalo semen with the same treatments though PTM was apparently high after 2 hr of equilibration (Table 2) These results show that the buffalo semen can be frozen successfully even without giving any holding time once the straws are cooled gradually to 5°C from 30°C within 2 hr. However for cattle semen, a holding time of at least 2 hr was highly beneficial and essential for better post-thaw results. Several investigators have shown the optimum equilibration period of less than 6 hr which in earlier studies ranged from 6 to 18 hr or even more. A storage period at 5°C appears to be less important, if cooling of semen to 5°C is done slowly. (Foote, 1970; Wiggin and Almquist, 1975; Sahni and Mohan, 1988a). The best PTM and incubation survival of cattle and buffalo sperm have been reported with an equilibration period of 0.5 to 2 hr than the longer time by Wiggin and Almquist (1975), Aguirre *et al.* (1978), Raju and Rao (1983), Dharmadheeran and Jose (1986), Sahni and Mohan (1988[a]) and Mathur (1989). Whereas, Crabo *et al.* (1980), Tuli *et al.* (1981) and Motwani *et al.* (1986) reported 4-5 hrs of equilibration at 5°C to be better than shorter or longer period for buffalo semen. This is contrary to the present findings. The different thawing temperatures used, however, had almost similar trend for improvement of PTM in both the species following freezing of their semen after 0 or 2 hr of equilibration. Among the two-and three-way interactions studied, only the species x equilibration interaction was significant for PTM both at 0 and 1 hr of incubation and the species x thawing interaction for post-thaw incubation survival. It showed that an equilibration period of 2 hr was advantageous for cattle than buffalo semen. The effect of thawing rates on motility was more marked in buffalo (38 to 50%) than the cattle (40 to 49%) semen.

Effect of thawing temperatures : The three thawing temperatures employed had significant ($P < 0.01$) influence on PTM at 0 and 1 hr of incubation of cattle semen, but in buffalo semen, the effect on motility was significant ($P < 0.01$) only at immediate post-thawing. The motility increased significantly ($P < 0.01$) with increase in thawing temperatures from 40° to 60°C in both the species, but the thermoresistance/incubation test of thawed semen revealed rapid deterioration in buffalo sperm motility as compared to cattle semen at all thawing rates (Tables 2 , 3). Similar findings with regard to post thaw recovery rate of bovine semen at higher thawing temperatures (from 5° / 35° C to 90° C) have been reported earlier

(Robbins *et al*., 1972; Papa, 1982; Tuli *et al.* 1985; Rao *et al*, 1986; Sahni and Mohan 1988, Murlinath *et al*, 1990). Further, cattle semen thawed at 70° C survived cold shock test significantly better than that thawed at 40°C (Papa, 1982). The present findings, thus, support these observations. The tendency for straws to bend at one end, and a highly lethal effect on sperm observed at higher thawing temperatures (>80°C) with a little longer exposure time of 15-20 seconds is in agreement with the reports of Robbins *et al.* (1972), Wiggin and Almquist (1975) and Sahni and Mohan (1988^b). The findings of thermoresistance test showed that deterioration in motility was least in cattle semen thawed at a faster rate, but buffalo sperm appeared to be more sensitive to such a thermal stress (Table - 2), even though, it has been proved to be more resistant to cold shock (Tomar *et al*, 1966). The disparity noted between cattle and buffalo sperm motility, initially and at 0 & 1 hr of post-thawing may be attributed to inherent differences in the quality, energy kinetics and metabolic activity and the kind of protective agents (egg yolk, sugar) used in the diluent. Further studies with respect to freezability, post-thaw thermoresistance and fertility trials using variable composition of diluents, cooling/equilibration times and thawing rates should disclose the actual reason(s) for disparity observed between the two species.

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Table 1 : Mean percentage of initial, pre-freeze and post-thaw sperm motility of cattle and buffalo semen.

S. No.	Breed	Bull No.	Sperm Motility			
			Initial	Pre-freeze	Post-thaw O hour	Post-thaw 1 hour
1	Holstein-Friesian (HF)	H–594	51.67 ± 1.67^{ab}	72.50 ± 2.81^{ab}	40.00 ± 1.72^{a}	19.86 ± 2.30^{a}
		H–678	84.14 ± 2.71^{b}	76.67 ± 2.11^{b}	51.67 ± 2.77^{b}	31.11 ± 2.45^{b}
		H–701	79.17 ± 2.01^{a}	69.17 ± 2.45^{a}	44.03 ± 2.29^{a}	19.77 ± 1.82^{a}
		Average	$81.67 \pm 1.28^{*}$	$72.78 \pm 1.47^{*}$	45.23 ± 1.71^{NS}	$23.56 \pm 1.77^{**}$
2	Murrah Buffalo	M–1248	75.00 ± 1.83^{a}	67.50 ± 1.71^{b}	44.31 ± 2.95^{b}	8.56 ± 0.64^{b}
		M–1294	71.67 ± 1.62^{a}	61.67 ± 3.33^{a}	37.08 ± 2.26^{a}	4.85 ± 0.41^{a}
		M–1296	84.17 ± 3.27^{b}	74.17 ± 3.52^{c}	52.08 ± 2.01^{c}	18.33 ± 1.12^{c}
		Average	76.94 ± 1.81	67.76 ± 2.03	44.49 ± 1.99	10.71 ± 1.42

* ** significant at 5% and 1% level respectively. between species.

Means bearing common superscript do not differ significantly between bulls within species.

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Studies On Leakage Of Transaminases From Buffalo Spermatozoa In Relation To Deep-freezing In Certain Extenders

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ABSTRACT

Egg-yolk-citrate glycerol (EYCG), tris-yolk glycerol (TYG) and citric acid-whey glycerol (CAWG) extenders were compared for the leakage of glutamic oxaloacetic transminase (GOT) and glutamic pyruvate transaminase (GPT) enzymes from buffalo spermatozoa during different stages of deep-freezing. A total of 40 ejaculates from five mature buffalo bulls (eight from each bull), exhibiting more than 70% initial motility, were frozen in these extenders. Leakage of GOT and GPT was least in TYG and the most critical stage of freezing was between equilibration and 24 hours after freezing.

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Various extenders have been used for deep-freezing of buffalo semen by different workers with conflicting results. The efficacy of extenders used for freezing buffalo semen was mostly tested by post-thaw motility (PTM) apart from the fertility trials. Any trauma to sperm membrane due to cold shock, disruption caused by ice crystals or unfavourable concentration of extender's ingredients causes release of enzymes (Tuli *et al.*, 1982). Glutamic oxalo-acetic transaminase (GOT) and Glutamic pyruvate transaminase (GPT) are concerned with oxidative metabolism (Salisbury *et al*, 1978). Keeping in view the involvement of these enzymes in sperm metabolism, an attempt was made to study their (GOT and GPT) release under varied conditions of processing and freezing semen in three extenders : Egg yolk citrate glycerol (EYCG), Tris yolk glycerol (TYG) and Citric acid-whey glycerol (CAWG), with respect to the sperm survivability.

Materials And Methods

A total of 40 ejaculates from 5 Murrah buffalo bulls (8 per bull) were collected with A.V. (42-47°C) at weekly intervals. After recording physical characteristics, each ejaculate was evaluated for mass activity, motile sperm %, live sperm % and sperm concentration by standard procedures (Herman and Madden, 1952). Morphology of spermatozoa was studied and subjected to deep- freezing.

Three extenders, (i) Egg yolk citrate glycerol (EYCG) (ii) Tris yolk glycerol (TYG) and (iii) Citric acid whey glycerol (CAWG) were used as per Salisbury *et al.* (1941), Davis *et al.* (1963) and Ganguli *et al.* (1973). Penicillin G sodium (1000 I.U./ml) and streptomycin sulphate (1 mg/ml) were added to each extender and pH adjusted to 6.8.

Each ejaculate was divided into 4 fractions. Of these, 3 fractions were diluted separately in the three extenders studied. The dilution rate was calculated on the basis of sperm concentration, to have 40 million spermatozoa in 0.5 ml. The final concentration of glycerol was kept at 7% by volume, with 4 hours equilibration period and the semen was deep frozen as per Cassou (1976) technique.

The level of GOT nand GPT enzymes in the seminal plasma was measured in fresh semen (stage 1), after complete extension (stage 2), at equilibration (stage 3) and 24 hours (stage 4) and 7 days (stage 5) after freezing. To study enzyme levels in seminal plasma of fresh semen, one fraction of each ejaculate was diluted in normal saline (control). One ml each from control, extended and equilibrated semen was centrifuged at 3000 r.p.m. (g = 1300) for 15 min at room temperature and the supernatant (seminal plasma) used for enzyme assay. Similarly, 24 hours and 7 days after freezing, 2 straws from each extended semen were thawed at 37°C for 1 min., centrifuged and supernatant used for the enzyme assay. GOT and GPT were estimated as per Wootton (1964). The data were analysed by using F test (Snedecor and Cochran, 1967)

Results And Discussion

Enzyme Levels in Seminal Plasma : Fresh semen (stage 1) : The mean GOT and GPT values in the seminal plasma of buffalo bull (Tables 1, 2) were found to be higher than those reported by other workers (Chauhan and Srivastava, 1973; Dabas *et al*, 1982; Iqbal, 1987; Varshney *et al*, 1978) and this could be due to presence of varying proportions of ageing and decaying spermatozoa in ejaculates from different bulls, differences in testosterone level (Mann and Lutwak-Mann, 1981) and variations in secretions from accessory sexual glands (Pace and Graham, 1970).

After complete extension (stage 2) : The seminal plasma levels of GOT and GPT after complete extension increased 3.43 and 10.49% in EYCG, negligible and 8.29% in TYG and 0.36 and 31.46% in CAWG extenders as compared to the levels in the seminal plasma of fresh semen. However, Iqbal (1987) reported much higher increase in GOT (113.20%) and GPT (66.40%) levels in the seminal plasma after extension in TRIS extender. The differences in GOT and GPT levels between extenders and first two stages were non-significant (Table 3).

After equilibration period (stage 3) : The levels of GOT and GPT at stage 3 increased to 7.47 and 21.59%, 2.42 and 16.44%, 9.06 and 31.76% in EYCG, TYG and CAWG extenders, respectively, over stage 2. However, this increase in levels was non-significant. The difference in GOT and GPT levels was also non-significant between extenders.

After freezing (stages 4 and 5) : The differences in the levels and per cent increase in GOT and GPT between extenders and stages after 24 hours and 7 days of freezing were statistically significant ($P < 0.01$, Table 3).

A similar trend of enzyme increase was observed by Dhami *et al.* (1987), where the increase was 61.32% (GOT) and 67.70% (GPT) in seminal plasma between prefreezing and 7 days postfreezing in EYCG extender. The increase in enzyme levels in TYG extender was in close agreement with the findings of Tuli *et al.* (1982). However, Iqbal (1987) noted 153.90% GOT and 90.50% GPT increase in TYG extended semen between extension and shortly after freezing. In CAWG extender, Tuli *et al.* (1987) reported 58.60 and 45.04% increase in GOT and GPT levels between first extension and 15 minutes after freezing, whereas in the present study, the increase was higher between stages 3 and 4 and between stages 4 and 5. The variations in the increase of enzyme levels at various stages of freezing in three extenders could be due to the differences in the composition and pH of extenders,

glycerol levels used, equilibration period, rate of cooling, time of exposure for vapour freezing, handling of semen and the differences in permeability of spermatozoa,

The significant ($p < 0.01$) lower levels of GOT and GPT in TYG at stages 4 and 5 (Table 3) could be attributed to the maximum protective action of TYG to sperm membrane against cellular injury (Crabo *et al*, 1971), as compared to EYCG and CAWG extenders. These findings are supported by Chinnaiya *et al* (1979), Kakar and Anand (1984) and Tuli *et al*. (1982).

The Leakage of GOT and GPT was found to be significant ($P < 0.01$) (Table 3) from stage 3 to stage 4 in all the three extenders. This is attributed to the temperature shock and sperm

Table 1 : Mean levels of Glutamic OxaloacceticTransminase (GOT) in seminal plasma of neat buffalo semen and during deep freezing in various extenders. (n mol pyruvate formed/min./ mL)

S. No.	Buffalo Semen	BULL NO.					Overall Mean ± SE
		M-150	M-586	M-610	M-952	M-962	
1.	Fresh Seminal Plasma	632.8±2.51	514.7±2.32	974.3±2.01	689.5±1.56	601.7±1.92	602.6^{a}±29.15
2.	EYCG						
	After complete extension	569.5±3.56	973.3±1.47	613.9±2.56	683.5±2.18	712.5±2.33	623.33^{b}±33.14 (3.43%)
	After equilibration	709.5±1.12	703.1±2.51	568.2±6.48	642.9±3.15	717.6±2.33	668.3^{c}±28.29 (7.47%)
	24 hrs. post-freezing	1087.5±0.32	1031.3±2.55	986.4±1.99	1071.6±2.19	977.9±3.19	1030.9^{d}±21.98 (60.17%)
	7 days post-freezing	1473.5±4.16	1388.7±2.68	1420.5±5.21	1581.4±1.52	1631.1±2.37	1499.0^{e}±46.75 (77.68%)
3.	TYG						
	After complete extension	612.3±3.11	541.3±2.41	563.5±2.32	643.8±2.11	617.3±9.52	595.6^{f}±18.78 (nil)
	After equilibration	654.9±1.56	571.5±2.29	532.9±2.41	659.4±1.95	632.5±2.54	610.2^{g}±34.89 (2.42%)
	24 hrs. post-freezing	839.5±0.98	912.1±1.65	883.2±6.45	971.5±2.62	837.3±2.58	888.7^{h}±24.99 (46.22%)
	7 days post-freezing	1014.4±2.89	1281.9±1.56	1345.3±6.52	1241.2±2.58	1416.1±2.59	125^{i}.8±68.11 (61.58%)
4.	CAWG						
	After complete extension	672.5±0.15	599.3±2.99	583.4±9.53	585.3±1.68	583.4±3.56	604^{j}.8±17.19 (0.336%)
	After equilibration	716.3±1.37	693.5±3.12	612.9±2.56	673.5±2.63	600.7±2.82	659^{k}.4±22.59 (9.06%)
	24 hrs. post-freezing	971.5±2.99	1130.0±3.15	1060.9±3.11	1137.4±2.36	1226.3±2.81	1105^{l}.2±42.52 (73.98%)
	7 days post-freezing	1384.4±3.45	1454.9±3.62	1371.7±1.62	1506.5±2.45	1773.5±2.63	1498^{m}.2±73.04 (65.22%)

For treatments : CD = 130.84 ($P < 0.01$)

Between extenders : $h < d, h < l, i < e, i < m$ ($P < 0.01$)

Between stages : $c < d, g < h, k < l, d < e, h < i, l < m$ ($P < 0.01$)

cell injury associated with freezing (Crabo *et al*, 1971), changes in mitochondrial sheath with loss of proteins from mid-piece (Graham *et al*, 1974) and increase in cell membrane permeability with or without rupture (Roychoudhary *et al* , 1974). The present observations are in close agreement with the reports of Iqbal (1987) and Tuli *et al*, (1982). The levels of GOT and GPT in seminal plasma were significantly ($P < 0.01$) higher at stage 5 in comparison with the levels at stage 4 in all the extenders (Table 3) suggesting further leakage of enzymes during storage. This could be due to increase in the concentration of ageing and decaying sper-

Table 2 : Mean levels of Glutamic Pyruvate Transaminase (GPT) in seminal plasma of neat buffalo semen and during deep freezing in various extenders (n mol pyruvate formed/min./ml.)

S. No.	Buffalo Semen	BULL NO.					Overall Mean ± SE
		M-150	M-586	M-610	M-952	M-962	
1.	Fresh Seminal Plasma	63.90 ±2.88	69.5 ±1.54	78.3 ±3.66	54.5 ±4.11	53.6 ±1.98	63.96^{a} ±2.24 (10.49%)
2.	EYCG						
	After complete extension	65.3 ±2.18	71.9 ±2.15	87.4 ±3.77	59.6 ±4.18	68.9 ±4.25	70.62^{b} ±4.67 (21.59%)
	After equilibration	77.3 ±3.25	89.5 ±3.99	103.3 ±4.55	68.5 ±3.12	83.4 ±4.15	84.40^{c} ±5.86 (94.06%)
	24 hrs post-freezing	108.0 ±9.66	149.4 ±10.16	213.6 ±8.32	109.9 ±8.25	141.7 ±8.11	144.52^{d} ±19.15 (85.91%)
	7 days post-freezing	174.9 ±11.25	206.1 ±10.3	288.5 ±9.90	148.2 ±8.40	179.3 ±12.3	199.40^{e} ±24.09 (8.29%)
3.	TYG						
	After complete extension	59.13 ±2.52	63.5 ±3.68	83.4 ±2.12	63.3 ±3.98	76.4 ±3.77	69.20^{f} ±4.58 (16.44%)
	After equilibration	69.5 ±2.18	73.1 ±3.05	95.2 ±4.00	71.4 ±4.96	89.2 ±4.83	79.68^{g} ±5.23 (33.64%)
	24 hrs post-freezing	82.6 ±2.11	85.9 ±3.77	132.4 ±3.24	99.1 ±3.99	106.1 ±4.14	101.22^{h} ±8.89 (53.33%)
	7 days post-freezing	103.7 ±7.58	145.3 ±9.25	168.4 ±8.16	127.2 ±11.25	148.6 ±13.12	138.64^{i} ±10.91 (31.46%)
4.	CAWG						
	After complete extension	70.9 ±2.52	83.3 ±3.14	109.4 ±2.92	83.4 ±3.66	73.4 ±3.64	84.08^{j} ±6.82 (31.76%)
	After equilibration	85.6 ±2.11	103.1 ±1.62	174.2 ±2.42	84.1 ±2.56	74.5 ±3.68	104.30^{k} ±18.07 (31.76%)
	24 hrs post-freezing	100.3 ±3.85	132.2 ±4.12	204.1 ±5.63	153.3 ±5.92	183.9 ±6.12	154.76^{l} ±18.39 (79.03%)
	7 days post-freezing	181.9 ±11.01	248.3 ±12.62	312.4 ±9.88	206.4 ±10.82	216.9 ±11.32	233.18^{m} ±22.49 (122.70%)

For treatments : CD = 34.04 ($P < 0.01$)
CD = 25.54 ($P < 0.05$)

Between extenders : $h < d, h < l, i < e, i < m$ ($P < 0.01$)
Between Stages $c < d, d < e, h < i, k < l, l < m$ ($P < 0.01$)

matozoa in cryopreserved semen (Mann and Lutwak-Mann, 1981). Similar findings have been reported by Iqbal (1987) and Tuli *et al* (1982) during storage of semen in LN_2. On the basis of GOT and GPT leakage, it is concluded that TYG is the best out of the three extenders used for deep-freezing of buffalo semen. The most critical stage of freezing is between equilibration and 24 hours after freezing, where maximal leakage of enzyme was recorded.

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Table 3 : ANOVA of GOT and GPT enzyme levels in seminal plasma of neat buffalo bull semen and during deep freezing in various extenders.

S. No.	Source of Variation	df	Between Bulls	df	Between treatments (extenders and stages)	df	Error
1.	GOT						
	Sum of squares	4	99369.69	12	7036701.50	48	286010.41
	Mean sum of squares	4	24842.42	12	586391.79	48	5958.55
	'F' calculated	4	4.17**	12	98.41**	—	—
2.	GPT						
	Sum of squares	4	30383.76	12	171002.96	48	19360.73
	Mean sum of squares	4	7595.94	12	14250.25	48	403.35
	'F' calculated	4	18.83**	12	35.33**	—	—

** $P < 0.01$

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A Comparative Study On The Length Of Cooling And Equilibration Time On The Freezability Of Cattle Semen.

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ABSTRACT

Relative importance of various cooling (to 5°C) and equilibration periods (0 to 2 hrs) on freezing of cattle semen was studied. It was found that in semen diluted in egg yolk Tris glycerol at room temperature (32°C), the cooling time was less important as compared to equilibration period. Half hour equilibration period improved the post-thaw motility significantly even when semen was cooled in just 15 minutes. However, the best results were obtained in cooling the straws in two hours followed by equilibration period of another two hours.

* * *

Cooling of the diluted semen to 5°C and its subsequent equilibration constitute the two major time consuming steps in deep freezing of

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cattle semen. The relative importance of the length of the two steps in the efficiency of deep freezing process has been quite controversial. The earlier workers in this field have recommended longer cooling time as well as longer equilibration periods at 5°C. However, semen was successfully frozen directly after its cooling to 5°C without storing it at 5°C. (Jones, 1969; Foote, 1970; Jahnel, 1968; Sahni and Mohan, 1988 a,b), provided cooling to 5°C is done slowly.

The present study was aimed to further verify the relative importance of cooling time (to 5°C) and equilibration period (at 5°C) in cattle semen freezing process. The study constituted the fourth experiment of the project undertaken to develop some quick test-freezing methods for predicting the freezability of cattle semen (Mathur, 1989).

Materials and Methods

The experiment involved study of 30 ejaculates, 6 from each of the 5 HF bulls maintained under identical managemental conditions at IVRI Germ Plasm Centre. Ejaculates were collected by the standard AV method at biweekly frequency.

Immediately after collection and evaluation, the ejaculates were diluted at 30°C with Tris-glycerol-egg yolk dilutor (containing 20% egg yolk and 7% glycerol) to have 60 millions sperm/m. The diluted semen was filled and sealed in 32 French straws (0.5 ml). It was then subjected to 8 different cooling time to 5°C and equilibration period at 5°C, constituting 8 different treatments (T) thus :

1. *Treatment 1* (T_1) - involved placement of straws at '10°C height' followed by its cooling to 5°C in 15-20 mts in LN_2 vapour (LNV).

2. *Treatment 2* (T_2) - Same as T_1 but additionally the straws were equilibrated at 5°C for 30 mts in LNV itself after their cooling to 5°C.

3. *Treatment 3* (T_3) - involved placement of cotton-plug protected straws at 5°C in LNV for 15 mts in the Thermoware container.

4. *Treatment 4* (T_4) - involved placement of straws directly from 30°C to 10°C water, followed by its cooling to 5°C in 15 mts in freezing chamber of the refrigerator.

5. *Treatment 5* (T_5) - Same as T_4 except that the straws were equilibrated in refrigerator at 5°C for 30 mts.

6. *Treatment 6* (T_6) - involved cooling of straws from 30° to 5°C in two hours in freezing chember of the refrigerator (Sahni and Mohan 1988 a, b).

7. *Treatment 6* (T_7) - Same as T-6 but additionally the straws were equilibrated at 5°C in refrigerator for 30 mts.

8. *Treatment 8* (T_8) - involved cooling of straws from 30° to 5°C in two hrs in freezing chamber of the refrigerator followed by two hours of equilibration at 5°C in refrigerator.

T_1, T_3 and T_4 were selected from the results of the three earlier experiments conducted in relation to the development of some rapid test-freezing methods for predicting the freezability of cattle semen (Mathur, 1989).

After giving the above treatments, one straw per treatment was used for prefreeze evaluation while the remaining straws were frozen by the two-step freezing method of Jondet *et al* (1980). Thawing was done at 37°C for 30 seconds. After thawing one straw (per treatment) was used for PTM evaluation, dead sperm, abnormal sperm and normal acrosomal count; one straw (per treatment) was incubated at 37°C for 1 hr followed by assessment of post incubation motility and normal acrosomes. The remaining one straw (per treatment) was stored (in thawing water) at 5°C for 24 hrs for assessing post-ageing motility.

The statistical analysis was done as per Steel and Torrie (1981) at Micro-32 computer of the Institute.

Results and Discussion

Mean values for 30 ejaculates after giving different cooling and equilibration periods (T_1 to T_8) in terms of motility, dead sperm, abnormal sperm and normal acrosomes at prefreeze, post-thaw, post-incubation and post-ageing stages are presented in Table 1.

For PTM the differences between bulls and between treatments were highly significant ($P < 0.01$), the values (mentioned within brackets) being in the following sequence for the different treatments T_8 (40.00), T_7 (36.00), T_5 (34.20), T_6 (34.6), T_3 (28.73), T_2 (28.00), T_1 (27.83) and T_4 (21.96).However, the values did not differ significantly among T_5 to T_8. For all the other parameters also, freezability was best for treatment T_8.

Table 1: Mean values for different semen quality parameters after giving different treatments for cooling and equilibration time at different stages of processing / evaluation.

Semen quality parameter and stage of processing / evaluation	Overall average for different treatments							
	T_1	T_2	T_3	T4	T5	T6	T7	T8
1. Percent motility at different stages – a) Prefreeze	a 65.16 ± 1.42	b 55.83 ± 2.28	b 64.66 ± 2.73	a 64.66 ± 2.18	a 62.00 ± 1.89	a 65.16 1.66	a 65.00 ± 1.76	a 64.50 ± 1.86
b) Post-thaw	cd 27.83 ± 2.74	cd 28.00 ± 2.29	cd 28.73 ± 2.67	d 21.96 ± 2.40	abc 34.20 ± 3.14	abc 34.06 ± 2.68	ab 36.00 ± 2.46	a 40.00 ± 2.57
c) Post-incubation	defg 10.93 ± 1.96	def 14.03 ± 2.68	bcde 15.16 ± 2.56	fg 8.03 ± 1.57	bcd 15.83 ± 2.44	abc 19.36 ± 2.57	ab 19.66 ± 2.74	a 25.93 ± 3.01
d) Post-ageing	cdef 15.23 ± 2.36	bcde 14.33 ± 1.90	bcde 15.20 ± 2.40	f 10.20 ± 1.86	bc 20.06 ± 3.21	bcd 18.96 ± 2.55	ab 22.63 ± 2.78	a 27.36 ± 2.98
2. Percent dead sperm at different stages. a) Prefreeze	bc 31.13 ± 2.55	a 38.56 ± 2.58	a 38.83 ± 3.05	bc 30.30 ± 2.64	b 32.43 ± 2.72	cd 26.16 ± 2.33	bc 26.93 ± 2.38	bc 30.10 ± 2.34
b) Post-thaw	75.26 ± 2.73	74.06 ± 2.22	74.80 ± 2.28	81.06 ± 2.08	71.66 ± 3.06	68.00 ± 2.75	66.30 ± 2.05	59.33 ± 2.93
3. Percent abnormal sperm at different stages. a) Prefreeze	b 12.16 ± 0.62	ab 13.33 ± 0.69	ab 12.66 ± 0.63	a 13.90 ± 0.62	b 14.00 ± 0.64	b 12.20 ± 0.54	ab 12.24 ± 0.54	13.13 ± 0.52
b) Post-thaw	ab 15.26 ± 0.59	ab 16.00 ± 0.67	ab 15.43 ± 0.63	a 16.80 ± 0.68	a 16.46 ± 0.62	ab 14.70 ± 0.53	ab 14.83 ± 0.54	ab 15.20 ± 0.67
4. Percent normal acrosomes at different stages. a) Prefreeze	abc 72.96 ± 2.06	abc 75.26 ± 2.13	d 69.00 ± 1.95	d 68.36 ± 2.13	abcd 73.10 ± 2.46	ab 76.25 ± 1.95	a 76.86 ± 2.16	abc 74.60 ± 1.39
b) Post-thaw	b 43.83 ± 2.80	a 50.40 ± 2.42	b 44.03 ± 2.50	c 37.20 ± 2.84	b 42.53 ± 3.03	a 51.23 ± 2.23	a 51.73 ± 2.41	a 51.53 ± 2.46
c) Post-incubation	dc 18.23 ± 1.91	bc 26.43 ± 2.51	cd 21.10 ± 1.80	c 16.96 ± 1.84	cd 21.56 ± 1.99	bc 25.40 ± 2.13	ab 28.06 ± 2.31	a 31.53 ± 2.49

Note - Figures with same superscript indicate non-significant difference between them.

The treatments were placed into three groups to compare the effects of equilibration on the freezability results :-

Group I - T_1 & T_2; Group II - T_4 & T_5 and Group III - T_6, T_7 & T_8.

Treatments in each group had the same cooling time (to 5° C), but the first one was not given any equilibration at 5° C while the second / remaining treatments were given 30 mts (T_2, T_5 and T_7) or 2 hr (T_8), equilibration 5°C.

The comparative study of the freezability (in terms of motility, dead sperm and normal acrosomes) at different stages of processing revealed that while the incorporation of equilibration or increase of equilibration period at 5°C had shown deterioration in semen quality at prefreeze stage, it caused improvement in freezability at post-thaw, post-incubationn and post-ageing stages. It suggested that prefreeze semen quality is not an indieator of post-freeze semen quality. Further, the effects of equilibration are more pronounced after heat and cold-stress of the post-thaw semen.

Comparison of freezaility of T_4 with T_5 (Group II) showed that incorporation of just 30 mts equilibration period to semen cooled to 5°C in just 15 mts (T_5), improved the freezability significantly. Further, the average PTM of T_5 was not significantly different from T_6 which involved a cooling time of 2 hrs (vs 15 mts cooling time of T_5), but without any equilibration period. Thus by giving just 30 mts equilibration at 5°C there was non-significant difference in the freezaility of semen cooled to 5°C either in 15 mts or 2 hrs. This showed that cooling time to 5°C was less important as compared to equilibration at 5°C. For achieving better freezability, some equilibration at 5°C is essential after cooling semen to 5°C.This is in full agreement with the observations of Gilbert and Almquist (1978).

It is essential that optimum combinations of cooling time and equilibration period need to be further experimented with more emphasis on reducing the cooling time (to 5°C) and increasing the storage period at 5°C, prior to its deep freezing in LNV.

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Semen Characteristics Of Successive Ejaculates In Cross-bred Bucks

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ABSTRACT

Semen characteristics of successive ejaculates in Alpine x Saanen x Malabari cross-bred bucks ejaculated two times daily (Group I) and three times daily (Group II), for a continuous period of 90 days were studied. Semen colour varied from creamy to thin creamy, yellowish milky to thin yellowish milky in successive ejaculates. Volume, density, mass activity, motility and sperm concentration showed a decreasing trend in successive ejaculates. Dead sperm %, sperm abnormality %, pH and MBRT showed an increasing trend with successive ejaculates. In bucks ejaculated twice daily all the characters significantly varied between ejaculates except volume, motility %, dead sperm % and abnormal sperm %. In bucks ejaculated thrice daily, all the characters except the latter two differed significantly between ejaculates. Semen characteristics of all the ejaculates in both groups were well within normal limits.

* * *

Semen composition is variable between different species, individuals of the same species, and between individual ejaculates. Age, size, breed, season, nutrition and frequency of ejaculation are the major factors which influence semen characteristics. This study involved the evaluation of successive semen samples of cross-bred bucks ejaculated at a frequency of twice and thrice daily continuously for 90 days.

Materials And Methods

Six adult Alpine x Saanen x Malabari bucks aged 2 to 3 years, belonging to AICRP on Goats for Milk, Mannuthy, were selected for study. The bucks maintained under identical feeding and management were selected randomly forming 2 experimental groups of 3 bucks each.

Group I : Two successive ejaculates were taken daily for a continuous period of three months.

Group II : Three successive ejaculates were collected daily for a continuous period of three months.

The characteristics of ejaculates such as colour, volume, density, mass activity, motility, percentage of dead and abnormal sperms were estimated by standard procedures (Roberts, 1971). BDH pH indicator paper strips (range : 5.3 to 7 and 7 to 8.5) were used for determination of pH. Concentration was estimated with Bausch and Lomb Spectronic 20 Photo-colorimeter, using standard procedure (Perry, 1969). Methylene Blue reduction test (MBRT) was carried out as modified by Joseph and Nair (1988).

The data were statistically analysed as per Snedecor and Cochran (1967).

Results and Discussion

The colour of the semen was initially creamy, which gradually changed to thin creamy yellow to yellowish milky and to thin yellowish milky with successive ejaculates and days. In general, volume, density, mass activity,

motility and sperm concentration showed a decreasing trend in successive ejaculates. Dead sperm %, sperm abnormality %, pH and MBRT showed an increasing trend in successive ejaculates of bucks collected twice and thrice daily. Dead sperm % and sperm abnormality % were unaffected by number of ejaculations per day. All the semen characteristics were well within normal limits in all the ejaculates of group I and group II bucks.

Table 1 : Seminal attributes of bucks ejaculated twice daily.

S.No.	Semen Characteristics	1st ejaculate (E_1)	2nd ejaculate (E_2)	(tn-1)
1	Volume (ml)	0.52 ± 0.19 (273)	0.48 ± 0.19 (272)	1.25
2	Density (D)	2.88 ± 0.37 (270)	2.51 ± 0.41 (269)	11.02**
3	Mass activity (+)	3.48 ± 0.72 (271)	3.23 ± 0.75 (270)	4.23**
4	Motility (%)	80.80 ± 9.91 (269)	79.54 ± 11.26 (271)	1.64
5	pH	6.91 ± 0.14 (265)	7.00 ± 0.11 (267)	12.07**
6	Concentration (10^6/cmm)	1.97 ± 0.54 (210)	1.46 ± 0.41 (199)	11.08**
7	Dead (%)	8.58 ± 4.41 (173)	9.09 ± 7.91 (170)	0.77
8	Methylene blue reduction time (seconds)	188.21 ± 102.44	264.26 ± 182.81	2.7**
9	Total abnormal spermatozoa (%)	2.64 ± 1.20 (179)	3.01 ± 3.01 (179)	1.19

Figures in parenthesis denote number of observations
** $P < 0.01$

Table 2 : Seminal attributes of bucks ejaculated thrice daily.

S. No.	Seminal attributes	1st ejaculate (E_1)	2nd ejaculate (E_2)	3rd ejaculate (E_3)	F Value	
					Between Bucks	Between ejaculates within bucks
1.	Volume (ml)	0.45 ± 0.14^a (276)	0.43 ± 0.15^a (276)	0.40 ± 0.15^b (276)	8.92*	6.84**
2.	Density (D)	2.51 ± 0.46^a (274)	2.26 ± 0.47^b (274)	1.89 ± 0.41^c (275)	0.54	63.28**
3.	Mass activity (+)	3.00 ± 0.70^a (275)	2.95 ± 0.80^a (271)	2.66 ± 0.82^b (274)	1.62	5.97**
4.	Motility (%)	78.39 ± 11.62^a (275)	76.92 ± 15.25^a (273)	72.64 ± 18.54^b (274)	4.91	3.59**
5.	pH	7.05 ± 0.14^a (269)	7.09 ± 0.13^b (267)	7.16 ± 0.13^c (270)	7.50	62.48**
6.	Concentration (x 10^6/cmm)	1.55 ± 0.48^a (213)	1.32 ± 0.40^b (211)	1.03 ± 0.31^c (205)	0.85	44.02**
7.	Dead (%)	8.38 ± 4.17 (168)	8.68 ± 7.32 (162)	9.30 ± 6.81 (165)	0.81	1.00
8.	MBR time (seconds)	259.08 ± 151.60^a (103)	293.85 ± 152.35^b (96)	404.15 ± 197.39^c (67)	0.85	14.62**
9.	Total abnormal spermatozoa (%)	2.73 ± 3.60 (179)	2.36 ± 2.40 (179)	2.92 ± 3.27 (179)	23.60**	0.15

Figures in parenthesis denote the number of observations.
* $P < 0.05$ ** $P < 0.01$ Figures with different superscripts are signficantly different.

Kastyak (1962) collected semen from ram thrice daily for a period of 25 days and reported that the volume of second ejaculate was larger and that of third ejaculate smaller than that of the first ejaculate. The present findings in bucks differ from that of Kastyak (1962) as volume showed a decreasing trend from first to third ejaculates.

Danov *et al.* (1967) opined that in rams with four successive ejaculations every other day, the ejaculate, volume and sperm concentration decreased considerably, sperm resistance and motility decreased insignificantly and seminal pH increased with the number of ejaculations. The present findings are in agreement with the findings of Danov *et al* (1967) except that the decrease in semen volume with successive ejaculates which was statistically insignificant.

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Effect Of Extenders On Quality Of Frozen Goat Semen*

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There is diversity of opinion about the best extender for freezing of buck semen. The combinations of 6% glycerol and 4 hours of equilibration in tris and skim milk extenders and 6% glycerol and 6 hours of equilibration in EYC extender were found to be better for freezing of buck semen (Sinha, 1989). The present study was made to find out the relative efficacy of tris, skim milk and EYC extenders for freezing of buck semen. A total of 25 ejaculates comprising 5 from each of the 5 bucks (4 Beetal and 1 crossbred, collected using AV) studied. Imme-

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diately after collection the semen samples obtained from 5 bucks were pooled together, split into 3 parts and washed twice using buffer solutions (Deka and Rao, 1984). The split sample washed using tris buffer was extended using tris extender (Foote, 1970), while the split samples washed using sodium citrate buffer (2.9%) were extended using skim milk (Rajkonwar *et al*, 1977) and EYC (Mathew, 1974) extenders. The washed semen was extended (1:5) at 37°C using non-glycerolated fraction (NGF) of the extender. For extension, the volume of semen prior to washing was considered. The glycerolated fraction of the extenders (volume = NGF) containing 12% glycerol was added to the primary extended semen at 5°C in 2 steps, at 15 minutes interval. The equilibration period at 5°C was 4 hours for the semen samples extended in tris and skim milk extenders and 6 hours for the semen sample extended in EYC extender. The semen was frozen in French straws (0.5 ml). The freezing and thawing were done as per Deka and Rao (1984). The semen was evaluated after equilibration and freezing for sperm motility by conventional method and for percentage of intact acrosomes by Giemsa staining technique (Watson, 1975). The analysis of the data was made after angular transformation of the percentages as per Snedecor and Cochran (1967).

The mean sperm motility after freezing in tris, skim milk and EYC extenders was found to be 65.20 ± 0.80, 56.02 ± 1.87 and 47.99 ± 2.00% respectively. The corresponding values for intact acrosomes were 79.01 ± 0.44, 55.80 ± 0.38 and 51.90 ± 0.54%. Sperm motility after freezing and the percentage of intact acrosomes after equilibration and freezing, varied significantly ($P < 0.01$) between extenders. The critical difference test showed that the mean sperm motility and percentage of intact acrosomes after freezing, were significantly higher ($P < 0.05$) in tris extender than in skim milk extender and in skim milk extender than in EYC extender. Similar observations were recorded by Deka and Rao (1985). On the contrary, post thawing sperm motility (PTM) was reported to be higher in EYC extender than in skim milk extender by Goffaux and Corteel (1967). This variation could be due to the differences in processing of semen and the composition of the extender. In the present study the seminal plasma was removed by washing which might improve the efficiency of the extender.

It is evident from the study that tris extender is superior to skim milk and EYC extenders for freezing of buck semen.

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Preservation Of Native Boar Semen*

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ABSTRACT

A total of 32 ejaculates (4 each) of 8 native boars were preserved in four diluents : EYGC, EYGB, Tris and GPSE at two temperatures (5° and 15°C). The keeping quality (KQ) was best in GPSE dilluent at 15°C, even after 96 hours of preservation. The poorest was EYGC diluent at 15°C. Overall progressive motility in all the diluents was superior at 15°C storage.

* * *

Scanty literature on the preservation of native boar semen is available. Hence, the present investigation was undertaken to compare the efficacy of certain diluents for preservation of native boar semen at two temperatures. (5°C and 15°C).

Materials and Methods

Eight native boars aged 14 to 24 months, maintained at AICRP on Pigs, Veterinary College, Tirupati, were used for this study. A total of 32 ejaculates, 4 from each boar were collected at weekly interval and preserved at 5° and 15°C by split technique in 4 diluents.

(1) Egg yolk glucose-glycine citrate (EYGC) (Tomar *et al.* 1969) (2) Egg yolk-glucose-sodium bicarbonate diluent (EYGB) (Kampschmidt *et al*, 1953) (3) Tris dilluent (Davis *et al*, 1963) and (4) Glucose potassium sodium tartrate sodium citrate dihydrate Edate (GPSE) (Tamuli *et al*, 1986).

The diluents were prepared aseptically and fortified with 1,000 units of penicillin and 1,000 mg of streptomycin per ml of the diluent.

Semen was diluted in 1 : 2 ratio. Progressive motility was assessed in all the samples immediately after dilution and after 12, 24, 48, 72, 84 and 96 hours of storage.

Results and Discussion

The percentage of progressively motile sperm was significantly higher ($P < 0.01$) in the diluents kept at 15°C, than at 5°C. The best results were obtained in GPSE followed by Tris, EYGB and EYGC diluents at 15°C (Fig 1). In GPSE diluent the overall motility was found to

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be 70% at 15°C. Similar observation was also reported by Tamuli *et al.* (1986) using the same diluent. The values obtained for the sperm motility in EYGB and EYGC were 57 and 50%, respectively. These findings are similar to those observed by Murthy and Rao (1975) with Large White Yorkshire semen using the same diluent.

The variability in percentage of prograssively motile sperm during storage between individual boars, between diluents and between preservation times (PT) was found to be significant ($P < 0.01$). The highest percentage of progressively motile sperm was found to be in GPSE (70%) and the least in the EYGC diluent (47.38) (Fig 2). No difference was noticed in the keeping quality of semen between EYGB and in EYGC.

The interaction due to diluent × PT, diluent × temperature, PT × temperature, diluent × PT × temperature was observed to be significant ($P < 0.01$). At 12 and 24 hours of preservation, the GPSE and Tris diluents showed better sperm motility when compared with the other diluents. The GPSE diluent was found to be better even at 72 hours of preservation with 62.3% sperm motility.

The superiority of the GPSE diluent might be due to the presence of glucose as well as sodium salt of EDTA. Glucose in the diluent acts as a substrate to support the viability of spermatozoa, maintains the osmotic balance and obliterates the harmful effects of diluent. The EDTA (Ethylene diamine tetra acetic acid), either depresses the enzymatic action of the proteases (Plisko, 1966) or prevents the decrease of aldolase enzyme action (Kurilo, 1968), thereby preserving the motility of spermatozoa.

Slightly higher percentage of motile sperms in Tris diluent (65.75%), as compared to EYGB diluents might be due to its ability to diffuse readily in the sperm cells and serve as an intracellular buffer (Bartlett and Van Demark, 1961).

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A Note On The Semen Characteristics Of Ganjam Bucks

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Five Ganjam bucks of 2-4 years age at Goat Breeding Farm, OUAT, Bhubaneshwar, reared under standard feeding and management practices were used. Fifty semen samples, ten from each buck, were collected by modified AV as per Rath *et al* (1971). The modified collection tube was insulted by putting a rubber condom containing warm water (40°C) over it to prevent cold shock.

The reaction time of Ganjam bucks (9.62 ± 0.97 sec) was comparatively less than that of Malbari (Patil and Raja, 1978), Black Bengal and Sannen (Sinha and Singh, 1985) bucks, indicating that Ganjam bucks are quick at service. The average volume of the ejaculate (0.94 ± 0.24 ml) almost confirms the findings in other breeds. However, Singh *et al* (1982) recorded little higher volume of semen in Jamunapari and Barbari bucks. The variations in the volume of semen may be due to breed characteristics and other environmental factors.

The mass activity (4.12 ± 0.05) and motility (75 to 80%) are in accordance with the observations of Singh *et al* (1982) and Borgohain *et al* (1985) in different Indian goat breeds. Sperm concentration (2309.17 ± 95.89 × 10^6/ml) in Ganjam buck semen is allied to that in Barbari, Jamunapari, Black Bengal and Sannen bucks, but differs from the reports of Kurian and Raja (1957) in Malbari bucks and Borgohain *et al.* (1985) in local goats of Assam. The lower sperm concentration of Ganjam bucks may be due to higher volume of semen. The percentage of live sperm (84.83 ± 1.02) is in close proximity with observations made in other breeds of goats and total sperm abnormalities (5.42 ± 0.31%).

The semen of Ganjam bucks is comparable to that of other breed of Indian goats for use in artificial insemination.

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A Note On Microbial Flora Of Buck Semen

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Contamination of fresh and preserved semen poses a great threat to successful breeding programme since it may lead to rapid decline in sperm motility (Sakala *et al*, 1961 and Roberts, 1971). Though reports on the microbial flora of bovine semen were available, literature on the buck semen is scanty (Sharma and Deka, 1986). Hence, the present work was undertaken to study the bacterial load and types of microbial flora present in the buck semen under normal conditions.

Two semen samples from each of the six bucks were obtained, before and after washing of the prepuce with sterile normal saline. Standard plate count method (SPC) was carried out by mixing sterile medium with serial tenfold dilution of the semen (10^1 to 10^4) to determine the total count of bacteria. The primary isolation was done on yeast extract agar. The isolates were identified as per Cruickshank *et al* (1974) and Buchanan and Gibbons (1974).

The mean number of colonies in the semen samples collected prior to washing of prepuce were found to be 80.9, 34.5 and 13.2 at 10^{-2}, 10^{-3} and 10^{-4} respectively, whereas, subsequent to prepucial washing, these were found to be 36.5, 19.1 and 12.8 at the same dilution rates. The mean percentage of reduction in the number of colonies consequent to prepucial washings was found to be 45, 55 and 94 respectively in the said dilutions. A total of 80 isolates were obtained in the semen samples collected before and after washing the prepuce with sterile normal saline.

The most common contaminants in the semen samples were found to be the *Bacillus* sp. followed by *Hafnia* sp. *Escherichia coli* and *Corynebacterium pyogenes*. Similarly, the prepucial washings also contained same species of bacteria. Sharma and Deka (1986) also reported isolates of *Proteus* sp. in higher numbers followed by *Bacillus* sp., *Diphtheroid* sp. and *Escherichia coli* in the frozen semen samples of bucks.

The presence of all the organisms both in prepucial washings and in semen indicated that semen was contaminated mainly due to infected prepuce. The decrease in the plate counts noticed in semen samples obtained after prepucialwashings could be attributed to the prevention of bacterial contamination consequent to washing of prepuce.

The *Coliforms* and *Diphtheroids* found in very small numbers in the semen samples collected after washing the prepuce, indicated that these contaminats can be eliminated by washing the prepuce prior to semen collection. Though these organisms do not have definite pathogenic significance, their increasing load may affect the motility and viability of sperms in the semen.

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Post-Partum Fertile Heat Period In Sahiwal x Jersey Cross-bred Cows With Three Levels Of Exotic Inheritance

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ABSTRACT

Studies on post-partum fertile heat were carried out on 55 Sahiwal Cross-bred Cows with three levels of Jersey exotic inheritance. The results indicated that J × S with 75% exotic inheritance had better reproductive performance regarding post- partum fertile heat as compared to J × S half-breds and J × S with 62.5% exotic inheritance. The effect of parity groups had highly significant effect on post-partum fertile heat.

* * *

Post-partum fertile heat is an important criteria of bovine reproductive efficiency. The present study deals with effect of genetic groups, parity groups and farms on post-partum fertile heat in Sahiwal x Jersey Cross-bred cows.

Material and Methods

A total of 55 cross-bred cows of the cattle breeding farm of Veterinary College and Agriculture College Dairy Farm, Nagpur were grouped on the basis of their exotic inheritance, thus : Group - 1 (50%) - 35 cows; Group 2 (62.5%) 10 cows and Group 3 (75%) - 10 cows.

Post-partum fertile heat was recorded as the time interval between parturition and subsequent fertile insemiation. Pregnancy diagnosis was done by gynaeco-clinical examination 60 days post- insemination. Confirmed pregnant cases alone were included in these studies. Observations were recorded for all the three levels of Jersey inheritance studied in respect of both the farms.

The cross-bred cows were divided into three parity groups comprising of parity Group

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I of primipara; Group II comprising of 2nd to 4th calvers and Group III comprising of 5th calvers and above.

The data was statistically analysed by applying least square analysis of variance design to study the effect of genetic groups, parity groups and farms on post-partum fertile heat.

Results and Discussion

The results indicated that, average interval to post-partum fertile heat was lowest (150.10 ± 48.75 days) in J × S with 75% exotic inheritance, followed by J × S half-breds. (171.17 ± 15.57 days) and highest in J x S with 62.5% exotic inheritance (208.70 ± 27.99 days).

Table 1 : Least square analysis of variance for post-partum fertile heat.

Source of Variation	d.f.	Mean-Squares
Genetic Groups	2	8452.40 N.S.
Parity Groups	2	28582.14**
Farm	1	1515.99 N.S.
Genetic group × Parity Group	4	2323.39 N.S.
Genetic group × Farm	2	3633.79 N.S.
Parity group × Farm	2	8746.18 N.S.
Residual	41	6392.54 N.S.

** P < 0.01 N.S. Non-significant.

The least square analysis of variance for post-partum fertile heat indicated that the difference between J x S cross-breds with three genetic groups was non-significant (Table - 1). These findings are in agreement with that of Choudhary *et al* (1977) who found no influence of genetic group on service period in Friesian x Sahiwal cross-bred Cows.

The average post-partum fertile heat interval was lowest (153.46 ± 14.38 days) in parity Group II, followed by 191.50 ± 50.53 days in parity Group III, with the highest average post-partum fertile heat interval (200.93 ± 22.12 days) in parity Group I. The difference between the three parity groups (Table 1) was found to be highly significant (P < 0.01). Malik and Sandhu (1968) observed the same trend in Sahiwal cows, and Das *et al* (1987) recorded that lactation order had a significant effect on service period in Jersey cows. However, these differ from Choudhary *et al* (1977), who recorded that parity group did not influence the service period in HF × Sahiwal half-bred cows.

Least square analysis of variance indicated no effect of farms on post-partum fertile heat. The interaction between genetic groups and parity groups and genetic group and farm was non-significant.

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Interaction Of Gonadotrophin Releasing Hormone And Oestradiol On Pituitary And Ovarian Responsiveness In Anoestrous Cows

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ABSTRACT

Pre-treatment of post-partum anoestrous cows with oestradiol-17B selectively increased the LH release, but not FSH in response to GnRH, while oestradiol alone failed to elicit any gonadotrophic response. Although all the GnRH treated cows responded with ovulation, a greater proportion of cows given oestradiol prior to GnRH responded with normal luteal function subsequent to ovulation. This response was not related to LH response. Cows with the greatest induced LH release had deficient progesterone function and vice-versa. The FSH release, however, was not related to the luteal function.

* * *

The ability of a single injection of GnRH to induce a pre- ovulatory type surge release of LH and FSH and ovulation in cows (Zolman *et al*, 1974) has led to its therapeutic use for induction of ovulation and ovarian function in post-partum cows. However, the results are variable and the induced cycles are often of shorter duration due to deficient luteal function (Kesler *et al*, 1978).

Oestradiol-17B(E_2) triggers the preovulatory surge release of gonadotrophins in naturally cycling cows. Exogenous E_2 is also shown to augment the pituitary responsiveness to release LH and FSH in response to exogenous GnRH (Kasner *et al*, 1981), but its effect on ovulation and subsequent luteal function is not known.

The present study was designed to investigate the changes in the pituitary responsiveness to release LH and FSH and their effects on the ovarian responsiveness in anoestrous cows treated with GnRH alone or in conjunction with E_2 and to examine the hormonal profiles that might account for normal luteal function.

Materials And Methods

The study involved 16 post-partum (38 to 62 days) crossbred anoestrous cows with inactive ovaries. The treatments consisted of a single I.M. injection of 250 μg GnRH (Factrel, Ayerst Lab., Canada) Group 1 (n = 5), 1 mg. Oestradiol – 17β (Sigma chemical Co., USA) in arachis oil followed 6h later by 250 μg. GnRH (Group 2, n = 7) and 1 mg Oestradiol-17β (Group 3, n = 4)

Animals were checked for oestrus, while ovulation and luteal function were monitored by rectal palpation and determination of serum progesterone (P_4) concentrations.

Blood samples were collected twice weekly, 2 weeks before and 6 weeks after treatment for P4 and at 0.5 h intervals for 2 h before and 8 h after administration of GnRH, at 1 and 2 h intervals till 12 h and 24 h for LH and FSH determinations. E_2 was estimated from 2 hourly samples. The samples were allowed to clot at 4°C and the serum was stored at - 20° C until assay.

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Progesterone was quantified by the RIA method as per Connor *et al* (1976). The mean extraction efficiency was 88% and the sensitivity was 55 pg/ml. The intra-and inter-assay C.V.% was 3 and 10% respectively.

Oestradiol-17β was determined by the RIA method used by Yu *et al* (1974), but without column chromatography. The mean extraction efficiency was 66.5%, while sensitivity was 2 pg/ml. The intra-and inter-assay C.V.% were 5 and 15%.

The LH and FSH concentrations were measured as described earlier (Narasimha Rao *et al*, 1988).

The endocrine data were analysed by the split-plot analysis of variance (Gill and Hafs, 1971). Student's 't' test and correlations were done as per standard methods. A computer model was used to estimate the area under the response curve.

Results and Discussion

Ovarian response : Following treatment, all the cows in groups 1 and 2 responded with ovulation and formation of luteal structures on the ovary. Two of 5 cows in group 1 and 5 of 7 in group 2, displayed oestrous cycles of normal length (20 ± 1.86 days), while the rest had initial short cycles lasting for 10 to 12 days. Spontaneous cycles ensued in all the cows. Overt oestrus was absent. None of the cows in group 3 responded to treatment.

Endoerine response : (Table 1)

Serum LH and FSH : Injection of GnRH caused an acute surge release of LH from the adenohypophysis resulting in significant ($P < 0.01$) elevation of mean serum LH level for 6.7 ± 0.45 h. Significant ($P < 0.01$) and synchronous rise in FSH was also seen but the response was less distinct.

The E_2 pre-treatment (group 2) selectively increased the pituitary responsiveness to GnRH with a greater ($P < 0.01$) magnitude of LH release but not FSH release, compared to single GnRH.

In the cows given E_2 alone (Group 3), the serum LH and FSH were unaltered. The data agree with those from previous reports on the GnRH induced LH and FSH release in anoestrous cows (Kesner and Convey, 1982) and on the potentiating effect of E_2 on the pituitary to release higher LH (Kesner *et al*, 1981), but not FSH (Padmanabhan and Convey, 1981) in response to GnRH in cows.

Serum Progesterone : The serum P4 levels increased for variable periods following treatment with a typical profile of normal cycle seen in 2 and 5 cows in groups 1 and 2, characterised by a gradual elevation beginning 3 to 4 days after treatment to maximum concentrations around day 10, and declined in about a week to the lowest levels.

The remaining 3 and 2 cows in the two groups displayed short term elevation with a significantly lower ($P < 0.05$) maximum concentration around day 6 to 7 and fell to basal levels around days 8 to 10. The cows in group 3, had basal levels of P_4 (0..48 ± 0.04 ng/ml) throughout the sampling period.

The occurrence of transient luteal responses to GnRH is consistent with other reports (Britt *et al*, 1975). However, the beneficial effect of E_2 pre-treatment in prolonging the life span of induced corpora lutea seen in this study has not been reported earlier.

Relationship of hormones : To elucidate the relationship of hormones and the endocrine milieu conducive for normal luteal function, the data were analysed in relation to normal

and short cycles (Table 2). The cows that responsed with normal cycles had a considerably lower LH peak height, duration and area compared to that in cows showing short cycles. A full LH release is not essential to induce ovulation and normal luteal function, as evident in two cows (one each in groups 1 & 2) with cycles of normal length had lower LH peak height (1.32 and 4.43 ng/ml), duration (4 and 4.5 h) and area (4 and 18 units). Conversely, the two cows which had highest LH response in groups 1 and 2 (peak 18.34 and 57.80 ng/ml, duration 8 and 9 h, areas 94 and 232 units) had short cycles lasting for 12 and 8 days respectively. The cows within treatment groups that had higher LH response also showed a tendency for deficient P_4 response.

FSH response, on the other hand, exerted no influence on ovarian function. The serum E_2 at the time of GnRH injection was relatively higher in the normal cyclic group. The correlations between LH and FSH peaks, E_2 and LH peaks were positive and significant ($P < 0.01$), while that between LH area and maximum P_4 was negative ($P < 0.05$).

The data are congruous with that of Lee *et al* (1985) who found higher LH response to GnRH in infertile cows at the time of breeding, probably reflecting pituitary and /or ovarian dysfunctions. Extremely high LH could result in desensitization of granulosa cells and their subsequent P_4 production (Kirchik and Birnbaumer, 1983).

The results further demonstrate that although E_2 pretreatment augmented the pituitary responsiveness to GnRH for LH release, this is not associated with increased ovarian responsiveness and contrarily, the induced LH and P_4 concentrations are inversely related. Nonetheless, a greater proportion of E_2 pretreated cows had induced corpora lutea with normal life span and level of function

Table 1 : Induced LH, FSH and Progesterone profiles following treatment of anoestrous cows with GnRH and / or oestradiol – 17 B

Parameter	Treatments		
	Group I 250 μg GnRH	Group II 1 mg E_2 + 250 μg GnRH	Group III 1 mg E_2
No. of cows	5	7	4
Preinjection concentration :			
LH (ng / ml)	0.33 ± 0.05	0.27 ± 0.03	0.38 ± 0.05
FSH (ng / ml)	0.41 ± 0.06	0.34 ± 0.07	0.32 ± 0.07
P4 (ng / ml)	0.38 ± 0.03	0.42 ± 0.04	0.48 ± 0.04
LH response :			
Peak height (ng / ml)	8.77 ± 3.28[a]	24.85 ± 6.53[c]	0.31 ± 0.08
Interval to peak(m)	156 ± 1.36	124 ± 0.56	–
Duration (h)	6.7 ± 0.45	7.9 ± 0.18	–
Total LH area (units)	46 ± 12.36[a]	110 ± 16.87[c]	–
FSH response :			
Peak height (ng / ml)	1.95 ± 0.82	2.62 ± 1.21	0.43 ± 0.09
Interval to peak (m)	126 ± 1.02	150 ± 1.22	–
Duration (h)	4.0 ± 0.12	5.5 ± 0.17	–
Total FSH area (units)	10 ± 2.06	17 ± 1.69	–
Maximum P4 Concentration (ng /ml)	3.46 ± 0.62	2.87 ± 0.72	–

a c significant at 1% level.

which can be partly related to the effects of elevated E_2 levels on follicular maturation prior to induced ovulation.

Acknowledgements

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Table 2 : LH and FSH release characteristics in relation to luteal function in anoestrous cows.

Parameter	Luteal Function	
	Normal	Sub-normal
No. of cows	7	5
Oestrous cycle length (days)	21.1 ± 1.92[a]	9.6 ± 2.02[c]
E_2 (pg / ml) before / treatment	41.86 ± 2.02	32.40 ± 1.82
LH : Peak (ng / ml)	15.94 ± 4.82	21.24 ± 6.02
Duration (h)	6.80 ± 0.44	8.30 ± 0.52
Area under response curve (units)	74 ± 13.22	96 ± 15.82
FSH : Peak (ng / ml)	2.11 ± 0.94	2.66 ± 1.22
Duration (h)	4.80 ± 0.12	5.50 ± 0.14
Area under response curve (units)	13.6 ± 2.08	14.8 ± 1.04
Maximum P_4 (ng / ml)	3.86 ± 0.94[a]	2.08 ± 0.80[b]

a b significant at 5% level
a c significant at 1% level

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Total Serum Protein And Haemoglobin Content In Anoestrus Rural Crossbred Heifers

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ABSTRACT

Total serum protein (4.46 ± 0.12 gm%) and haemoglobin (7.92 ± 0.25 gm%) concentrations were significantly lower in experimental anestrus heifers suffering from heavy helminthic infestation and malnutrition than in normal control cyclic heifers. Better managemental practices with supplementation of trace elements for a period of 20 days resulted in significant increase in levels of total serum protein (7.30 ± 0.24 gm%) and haemoglobin (10.33 ± 0.28 gm%) in close approximation to those obtained in control normal cyclic heifers (7.20 ± 0.36 gm% and 11.92 ± 0.34 gm%)

* * *

Protein deficiency retards the development of the sex organs and body growth in young animals and affects subsequent reproductive performances (Herrick, 1977). Haemoglobin values might have some role in influencing the reproductive status of animals. Hansel (1955) reported low haemoglobin values in many anestrus animals.

Materials and Methods

Forty crossbred (Jersey × non descript local) heifers not coming to first estrus within the age of 36 months and 10 crossbred heifers exhibiting first estrus within the age of 18 to 24 months, were selected at random from adjoining villages and included in the present study. Faecal samples of each animal were examined as per Soulsby (1982) for 3 consecutive days to assess the extent of parasitic infestation. 20 ml. of blood was collected from each animal of control and experimental groups. 2 ml. of blood was taken in a sterilized dry test tube containing anticoagulant (Sodium flouride - 10 mg. per ml.) for estimation of haemoglobin, while, the serum sample from the remaining blood was collected as per the standard technique. Total serum protein (TSP) was estimated by the methods of Wootton (1974). Blood haemoglobin (Hgb) was estimated by Sahlis acid haematin method as per Sastry (1983). Thirty experimental anestrus heifers were subjected to remedial measures thus :

(a) *Deworming* : All the animals were given Levamisole Hcl. B.P. (Vet) 30% w/w @ 150 ml./ 100 Kg. body weight per animal after dissolving 10 gms. of the drug in 600 ml. of drinking water. 7 days later, Hexachlorothane B. Vet. C 85% w/w @ 10 gms. per 50 Kg. body weight per animal in 2 divided doses at an interval of 48 hours, was given.

(b) *Improvement of nutritional status* : Each animal after deworming was given 2 Kg. of concentrate mixture in the form of 'Epic' (a balanced cattle feed manufactured by the West

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Bengal Poultry and Dairy Development Corporation, Kalyani) and 209 gms. of germinated gram per day. Sufficient paddy straw and available greens along with clean drinking water were also provided. Oral additional feed supplementation for 20 days was given to each animal using "Vet's Trace Mineral (Cattle)" (Vets Farma Pvt. Ltd; Jalandhar - 144 001) containing 6 trace elements, @ 10 gms. per day.

Anestrus heifers which came into first behavioural estrus after this regime were again subjected to faecal examination and estimation of TSP and blood Hgb levels for comparison. The data obtained was subjected to statistical analysis as per Snedecor and Cochran (1967).

Results and Discussion

The mean values with standard errors (Table 1) for TSP and blood Hgb levels in control normal cyclic heifers were found to be higher (7.20 ± 0.36 gm% and 11.92 ± 0.34 gm%) than those obtained in experimental anestrus heifers (4.46 ± 0.12 gm% and 7.92 ± 0.25 gm%) whereas, the anestrus heifers subjected to treatment regime showed increased values in close approximation (7.30 ± 0.25 gm% and 10.33 ± 0.28 gm%) to those obtained in the control normal cyclic heifers. Analysis of variance (Table 2) indicated that the mean concentration of both TSP and blood Hgb had significant difference among the groups. It was also observed by critical difference test (Table 1) that the levels of both TSP and blood Hgb varied significantly between the normal cyclic and anestrus heifers and between the anestrus heifers and the treated anestrus heifers. The differences between the normal cyclic heifers and treated anestrus heifers were insignificant. The lower values of TSP and blood Hgb in anestrus heifers in comparison to the normal cyclic heifers in the present studies are in full agreement with the reports of Naidu and Rao (1982) and Chetty and Rao (1986). The TSP and Hgb deficiencies in the anestrus heifers studied, might be due to very poor animal husbandry practices, lack of de-worming programme and malnutrrition. All the experimental anestrus heifers were found to be heavily infested with *Strongyles*, *Amphistomes* and *Fasciola* Sp. in combination. Soulsby (1982) reported that serum protein and haemoglobin levels

Table 1 : Mean values with Standard Errors for levels of total serum protein (gm %) and blood haemoglobin (gm %) in different groups of crossbred heifers.

Blood constituent	Cyclic heifers	Anestrus heifers	Anestrus heifers which came into first estrus after better management and feeding
Total serum protein	7.28^{a} ± 0.36 (10)	4.46^{b} ± 0.12 (40)	7.30^{a} ± 0.24 (10)
Range	5.20 – 8.80	3.20 – 6.00	6.00 – 8.50
Blood haemoglobin	10.92^{a} ± 0.34 (10)	7.92^{b} ± 0.25 (40)	10.33^{a} ± 0.28 (10)
Range	10.20 – 13.62	5.75 – 12.00	9.00 – 11.00

Figures in the parenthesis indicate the number of observation.
Means having the same superscripts do not differ significantly ($P < 0.01$)

reduced significantly due to gastro- intestinal parasites and liver flukes. Significant increase in TSP and Hgb values in the anestrus heifers after corrective measures and treatment suggested that low TSP and low blood Hgb content might be responsible for causing anestrous condition in crossbred heifers, in association with heavy helminthic infestation as reviewed by Laing (1979) and Sane *et al* (1982).

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Table 2 : Analysis of variance of total serum protein and haemoglobin content in different groups of crossbred heifers.

Blood constituent	Source of Variance	d. f.	Mean Square (MS)
Serum Protein	Between groups	2	51.734**
	Within groups	57	0.7699
Blood haemoglobin	Between groups	2	74.762**
	Within groups	57	2.125

** = Significant at 1% level

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Serum Protein Levels Around Induced Oestrus In Buffaloes Following PGF_2-Alpha Administration

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ABSTRACT

The pattern of change in serum proteins concentration was estimated following induction of oestrus with PGF_2-alpha through intramuscular (IM 25 mg) and intra-vulvo submucosal (IVSM 8 mg) routes in buffaloes having 8-10 day old corpus luteum. No Significant change in protein, albumin and globulin concentrations was observed before, during and after oestrus in control (n = 6), IM (n = 14) and IVSM (n = 11) treated buffaloes.

* * *

Application of PGF_2-alpha in controlling oestrous cycle in buffaloes has not met with consistent success. Estimation of serum biochemical parameters might provide indications that could be helpful in understanding the actions of alien compounds which are expected to alter the blood biochemical milieu. The present communication reports an investigation to monitor changes in levels of serum protein and its fractions during normal as well as induced oestrus in buffaloes. Oestrus was induced using 25 mg and 8 mg of PGF_2-alpha administered through intra-muscular (I/M) and intra-vulvo-submucosal (IVSM) routes, respectively.

38 buffaloes belonging to the dairy Farm, Department of Animal Science, PAU, Ludhiana were utilized for the study. Of these 32 distributed equally in two groups were subjected to differential PGF_2-alpha (Dinolytic Vet. Upjohn Ltd, Crawley, Sussex, England) administration treated with 25 mg by I/M route in one group while the other group received 8 mg by IVSM route. 6 buffaloes in oestrus served as control (Group I). Oestrus was detected by vasectomized bull(s) and also rectal palpation. 14 (Group II) and 11 (Group III) buffaloes were detected in oestrus within 144 hours in I/M and IVSM treatment groups, respectively. Serum samples were collected at 24-hourly intervals, 3 days before and after the day of oestrus from all the 38 buffaloes and preserved at – 20°C pending analysis for total protein and albumin (Henry *et al*, 1957).

The levels of serum total protein, albumin and globulin did not differ significantly (Fig 1) between days around oestrus in control (group I) and both the induced groups (II and III). The difference in values between control and each of the treated groups on all the days of observation were non-significant, except that the difference between group I and II was significant ($P < 0.05$) on day 1 before induced oestrus. The differences in serum albumin concentration between group I and II were non significant on all the days of observation. Consistantly higher levels of serum albumin were recorded in IVSM induced oestrus buffaloes (group III) as compared to the control animals (group I), the difference being sig-

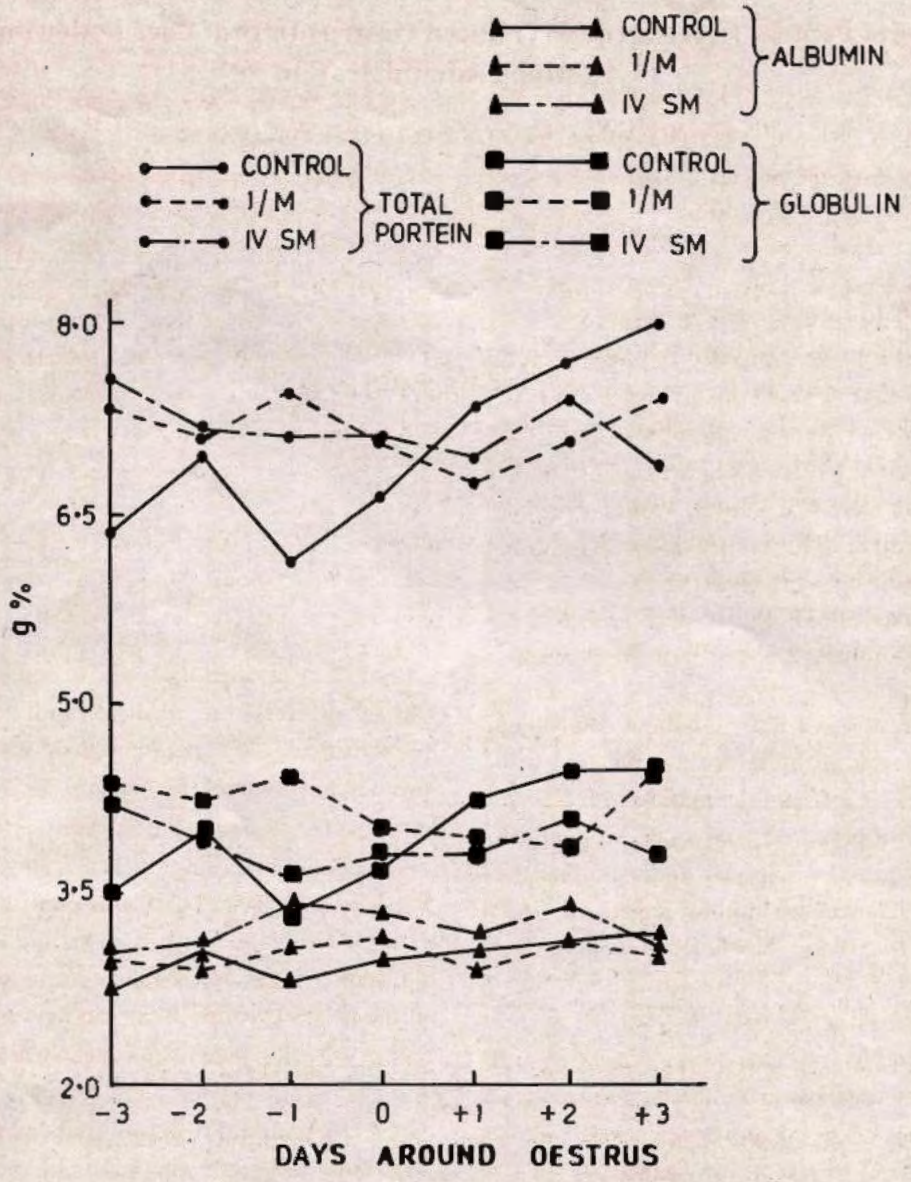

Fig. 1 MEAN TOTAL SERUM PROTEIN AND ITS FRACTIONS AROUND OESTRUS IN PGF_2 ALPHA INDUCED AND CONTROL BUFFALOES.

nificant only on day before oestrus. The value did not differ significantly between control and each of the induced groups on all the days of sampling, except that the difference between control (group I) and I/M treated group (II) was significant on day before induced oestrus.

The levels of serum total protein obtained before, during and after oestrus in control group are comparable with earlier reports in buffaloes (Kulkarni *et al*, 1984). Non significant difference in serum protein level within all treatment groups indicate that serum protein synthesis did not alter during different stages of oestrus implying that PGF_2-alpha administration had no effect on serum protein synthesis during manifested oestrus indicating localized rather than systemic action. Although Patil (1976) observed that an optimal protein level was required for oestrus expression, Jo *et al* (1982) found no difference in serum protein concentration between stages of the oestrous cycle in cows. However, Roychoudhry and Razdan (1965) observed considerable variation in the total protein content of cervical mucus at varying intervals of oestrus in the same animal and also between animals. Decline in protein level of cervical mucus has been reported in buffalo-heifers induced to oestrus following application of PGF_2-alpha (Prasad *et al*. 1981). This could be attributed to the excess of constitutent water in the thin and copious cervical mucus during oestrous phase which might have resulted in the lower values obtained in their studies. Significant variations in serum protein, albumin and globulin concentrations between control and treated groups only at certain intervals might be due to normal variations of the protein fractions independent of PGF_2-alpha influence. Present study indicates restricted action of PGF_2-alpha on serum biochemical milieu.

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Blood Glucose And Protein Levels In Crossbred Cows During Pregnancy.

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The present study was undertaken to determine level of blood glucose and serum protein during different periods of gestation in crossbred cows. Forty healthy crossbred cows (Jersey × Holstein) were included in the study. These animals were divided into Phase - I (first month to third month), Phase - II (fourth month to sixth month), Phase - III (seventh month to ninth month) and Phase - IV (One week post-partum). The parameters studied in each group with equal number of animals were levels of blood glucose (Nelson, 1945) and total serum protein (Wooton, 1974).

10 ml blood was collected from jugular vein of each animal. In early gestation, blood samples were collected during the Ist week after A.I., 2nd and 3rd month of pregnancy; in mid-gestation, during 4th, 5th and 6th month; in advanced gestation during 7th, 8th and 9th month of pregnancy and lastly on 7th day after parturition. A part of the blood was kept in vials containing sodium citrate (5 gm per ml) as anticoagulant and utilised for estimation of glucose. The remaining blood was allowed to clot in a big test tube and serum separated and utilised for biochemical estimation of total serum protein (TSP).

Results and Discussions

The blood glucose level decreased gradually from Ist (61.1 ± 1.07 mg%) to 6th month of pregnancy (53.2 ± 0.67 mg%). Thereafter, it increased progressively up to the day of parturition (59.4 ± 1.04 mg%). Again, a sudden fall in blood glucose level was observed one week after parturition. The mean TSP level in the first month of gestation was 7.2 ± 0.14 gm%. It increased slightly (7.5 ± 0.11 mg%) in 2nd month and decreased in the 3rd month (7.3 ± 0.09 gm%). From the third month onwards, serum protein concentration showed an increasing trend which continued till ninth month of gestation. Decreasing trend was observed on the day of parturition up to one week post-partum. Analysis of variance showed a highly significant variation ($P < 0.01$) of blood glucose and TSP concentrations between phases and between collections within phases of parturition.

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By least significance difference test it was observed that the mean blood glucose concentration of Phase - I differed significantly from other phases. There was significant difference between Phase - II and Phase - IV while Phase - III did not differ significantly from Phase II and Phase - IV. The rise of blood glucose level in advanced pregnancy (Phase - III) might be due to the release of glucocorticoid having a gluconeogenic and anti insulin effects. At post-partum period the low blood glucose level might be due to temporary decrease in plasma triglyceride (Sato, 1973).

All the four phases of gestation significantly differed from each other in respect of TSP concentration. The level was highest (8.6 ± 0.1 gm%) in advanced gestation and lowest (7.35 ± 0.07 gm%) in early gestation. Total protein in circulation represents a balance between the biosynthesis and catabolism or mechanical loss. Growing foetus draws nutrients and raw materials from dam's circulation which is also a source of gama globulins (Smith, 1962). TSP level was higher in late pregnancy compared to lactating Hariana cows (Dutta and Dugwekar, 1983). Findings of the present study are in agreement with those of Pathak *et al*, (1986) in Surti buffaloes. Variation in the early stage of gestation reveals its demand and utilization for the physiological events that take place during this period (McDonald, 1980). On the other hand, TSP concentration in the early four months of gestation was somewhat lower than in advanced gestation. This might be due to the fact that animals of early gestation were lactating and pregnant, whereas those of advanced pregnancy were dry and pregnant.

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Blood Serum Cholesterol In Pubertal Cycling And Non-Cycling Surti Buffaloes

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Cholesterol plays vital role in the physiology of reproduction (Taylor *et al*, 1966 and Singh *et al*, 1988). Present investigation was undertaken to know the sequential changes in the levels of cholesterol during estrous cycle and compare it with that of farm and rural anestrus buffalo heifers. Six normal cycling and six post-pubertal anestrus heifers of University farm, reared under standard nutritional and managemental practices were studied. All the heifers were of 18 mths. age, healthy, clinically normal with body weight around 250 kg.

Jugular blood samples were collected at 8 A.M. on alternate days from the day of estrus through complete estrous cycle over 12 different stages of 22 days (Table 1a). Such 20 complete estrous cycles were considered. Similarly blood samples were also collected from farm anestrus heifers for 22 days during this period and termed as 'corresponding stages' (Table 1b).

Apart from these, 48 single blood collections were made from anestrus heifers of six different villages around Anand centre averaging 8 animals per village. All the serum samples were stored at – 20°C till analysed. Total and free cholesterol were estimated by method of Schoenheimer and Sperry (1934). Statistical analysis of the data were as per Snedecor and Cochran (1971).

The results revealed that overall average value of total cholesterol for normal cycling heifers (168.93 ± 11.72 mg%) was significantly ($P < 0.05$) higher compared to that of the farm anestrus (147.54 ± 14.77 mg%) and village anestrus (144.72 ± 3.70 mg%) heifers. The levels of total cholesterol were at lower plateau for anestrus heifers compared to cycling heifers of farm (Table 1b, 1a).

The difference in the levels of free cholesterol due to the stages of estrous cycle was significant, while it was non- significant for corresponding stages of farm anestrus heifers. The average levels of free cholesterol for normal cycling, farm anestrus and village anestrus heifers were 17.30 ± 1.49; 19.51 ± 3.34 and 18.08 ± 1.01 mg% respectively. Statistically the difference between the levels of normal cycling and farm anestrus heifers was only significant ($P < 0.05$). The value of ester cholesterol showed almost same trend as that of the total cholesterol. The ratio of total is to free cholesterol was significantly higher for normal cycling heifers (11.80:1) compared to that of the village (8.9:1) and farm (8.9:1) anestrus heifers respectively.

Present findings are in agreement with that of Zala *et al*, (1972) for Surti and Luktuke *et al*, (1979) for Murrah buffaloes. These have also revealed low level of total cholesterol and poor

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mobilization of free form resulting into inadequate synthesis of sex steroids, which may be the probable reason for anestrus condition.

Increasing trend of total cholesterol during luteal phase indicates non-utilization of cholesterol and more synthesis as a require-

Table 1 : Blood serum cholesterol in normal cycling out anestrus Surti buffalo heifers ($\bar{X} \pm SE$)

Days of Oestrous cycle	Normal cycling (n = 20)				Anestrus condition (n = 6)			
	Cholesterol (mg %)			T : F Cholesterol Ratio	Cholesterol (mg %)			T : F Cholesterol Ratio
	Total	Free	Ester		Total	Free	Ester	
Day 0 (Heat)	144.98 ± 11.94	abc 18.65 ±2.00	128.34 ±12.29	10.82 ±1.85	128.75 ±11.93	20.04 ±1.53	108.71 ±12.43	6.68 ± 0.83
Day 2	160.97 ±11.09	cd 14.18 ±1.58	146.78 ±11.54	14.97 ±2.21	134.25 ±6.95	20.26 ±4.26	113.98 ±10.49	8.13 ± 1.63
Day 4	161.04 ±11.26	d 13.67 ±1.14	147.38 ±11.23	13.75 ±1.59	162.27 ±15.04	17.65 ±3.84	144.61 ±16.16	11.66 ± 3.16
Day 6	170.09 ±12.73	abcd 16.78 ±1.39	153.31 ±13.06	11.54 ±1.35	140.44 ±12.28	17.46 ±3.80	122.98 ±13.79	10.04 ± 2.09
Day 8	164.99 ±12.14	abcd 17.14 ±1.40	147.83 ±11.97	11.73 ±1.79	144.92 ±7.40	16.62 ±2.99	128.29 ±5.91	9.69 ± 1.22
Day 10	177.73 ±15.36	a 21.05 ±1.72	156.65 ±15.43	10.28 ±1.89	145.69 ±11.96	16.24 ±3.30	128.85 ±11.02	10.41 ± 1.57
Day 12	168.98 ±11.53	bcd 15.42 ±1.26	165.75 ±11.51	12.76 ±1.48	155.13 ±20.33	21.43 ±4.95	133.78 ±24.20	9.33 ± 2.12
Day 14	177.95 ±12.24	abcd 16.87 ±1.38	162.08 ±12.16	12.50 ±1.80	131.60 ±14.66	18.53 ±3.79	112.51 ±15.65	8.43 ± 1.64
Day 16	190.12 ±10.98	abcd 17.90 ±1.43	172.21 ±10.72	12.36 ±1.45	155.25 ±13.37	19.39 ±3.02	135.90 ±15.65	9.28 ± 1.75
Day 18	164.22 ±7.48	abcd 17.81 ±1.46	146.38 ±7.61	10.87 ±1.31	157.93 ±14.85	24.04 ±1.92	133.91 ±15.49	6.81 ± 0.87
Day 20	174.78 ±9.50	ab 19.68 ±1.84	155.05 ±9.84	10.24 ± 1.16	158.87 ±19.19	20.31 ±2.80	138.41 ±19.56	8.33 ± 1.62
Day 22	171.32 ±12.67	abcd 18.41 1.03	152.95 ±12.41	9.77 ±0.81	155.35 ±21.35	22.08 ±2.28	133.27 ±22.52	7.71 ± 1.74

Means with different superscripts differ significantly with each other at 5% level of significance.

ment either as a source of energy or as a precursor for steroid hormones (Patel, 1988). Free cholesterol showed increase toward early luteal phase followed by decline indicating mobilization of cholesterol to free form as a requirement and its subsequent utilization for biosynthesis of progesterone at peak luteal phase (Talavera *et al*, 1985). During the follicular phase, increase in cholesterol level may possibly be related to an increased biosynthesis of cholesterol rather to a difference in the rate of catabolism. Estrogen is also known for its influence of the complex relationship of pituitary-thyroid-adrenal function and thereby influences the cholesterol concentration (Bloch and Rittenberg, 1942). Estrogen has an effect on carbohydrate metabolism which in turn causes increased production of cholesterol in endocrine gland tissues from acetate (Shahukar *et al*., 1985). Shrivastava and Kharche (1986) and Singh *et al*. (1988) recorded similar results for blood cholesterol status in buffaloes.

Thus, fluctuations in the circulatory levels of cholesterol reflected its demand, synthesis and utilization during different reproductive phases.

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Serum Cholesterol Level In Crossbred Cows During Prepartum, Partum And Post-partum Period

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ABSTRACT

Pre-partum, partum and post-partum serum cholesterol levels were studied in 10 adult pluriparous and non-lactating Jersey crossbred cows. Serum Cholesterol level (mg%) declined significantly from 7th month of gestation (210.00 ± 4.39) till the day of parturition (102.00 ± 4.58) with increasing trend (147.50 ± 7.25) at one week after parturition.

* * *

Cholesterol is regarded as a widely distributed steroid precursor in blood in free and esterified forms. Hydrolysis of cholestered ester is essential for the synthesis of steroid based sex hormones (Nair *et al*, 1987). Therefore, the level of Serum cholesterol may have direct or indirect role in the process of parturition.

Materials and Methods

Ten adult pluriparous and non-lactating pregnant Jersey crossbred cows of identical 4 to 5 year age group, maintained under standard farm and management practices, were selected at random from the Govt. Dairy Farm, Haringhata, for the present study. Blood samples were taken from each animal at 7th, 8th, 9th month of pregnancy, on the day of parturition and one week after parturition and serum collected therefrom for bio-chemical estimation of serum cholesterol level as per the method of Henly (1957). Serum cholesterol levels were studied in 3 phases and 5 stages, thus :-

Phases	Stages
1 Phase I (Prepartum)	7th, 8th and 9th month of pregnancy
2 Phase II (Parturition)	Day of parturition
3 Phase III (Post-partum)	One week after parturition

The data obtained were subjected to statistical analysis as per Snedecor and Cochran (1967).

Results and Discussion

The results revealed that serum cholesterol level in Jersey crossbred cows started declining from the highest concentration (210.00 ± 4.39 mg%) at 7th month of gestation to its lowest value (102.00 ± 4.58 mg %) on the day of parturtition (Table 1). Thereafter, the receding value started increasing and was found to be 147.50 ± 7.25 mg% at one week after parturition. The average serum cholesterol level for the three stages of prepartum phases was recorded as 182.86 ± 20.20 mg%. Analysis of variance of serum cholesterol level (Table 2) showing the effect of times of sampling in prepartum, parturition and postpartum phases indicated an overall significant ($P < 0.05$) difference between times of sampling at various phases. Least significance difference test indicated that the level of serum cholesterol differed significantly ($P < 0.05$)

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between 7th and 9th month of gestation; between 7th month of gestation and day of parturition; between 8th month of gestation and day of parturition, and between 7th month of gestation and one week after parturition. On the contrary, it did not differ significantly between 7th and 8th month and between 8th and 9th month of gestation. The trend in respect of the fall in the level of serum cholesterol with advancement of pregnancy as recorded in the present study is in agreement with the findings of Jadhav *et al* (1977). According to Dindorkar *et al* (1984) thyroid hormone influenced cholesterogenesis during pregnancy. Possibly during progesterone synthesis in pregnancy, there may be an increased turnover of cholesterol from plasma pool. After parturition, the picture was reversed with a rising trend in the level of serum cholesterol. Stoeckl *et al*. (1975) observed that estrogens coupled with thyroxine might be mainly instrumental in reducing cholesterol concentration during advancing pregnancy. After parturition, the estrogen titre as well as thyroxine concentration decreased and when these inhibitions were removed, cholesterogenesis again gained momentum.

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Table 1 : Mean values with standard errors of serum cholesterol concentration (mg%) in crossbred cows.

	Prepartum			Parturition	Postpartum
	Month of gestation			Day of parturition	One week after parturition
	7th	8th	9th		
Mean	210.00^{a}	185.40^{ac}	153.20^{bc}	102.00^{b}	147.50^{bc}
± S.E	± 4.39(10)	± 5.30(10)	± 8.99(10)	± 4.56(10)	± 7.24(10)
Range	190 – 230	160 – 208	100 – 186	80 – 120	130 – 195

Mean ± S.E. of prepartum phase182.86 ± 20.20
Figures in the parenthesis indicate the number of observations.
The means having same superscripts or without any superscripts do not differ significantly ($P < 0.05$)

Table 2 : Analysis of variance of serum cholesterol level (mg %) in crossbred cows showing the effect of times of sampling in different phases studied.

Source of variation	d. f.	S. S.	M. S. S.	F
Times of sampling	4	67109.3	16777.325	9.033*
Error	45	83581.8	1857.370	–
Total	49	150691.1	–	–

* $P < 0.05$

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Efficacy Of PGF_2 alpha (Dinoprost) Using Two Routes Of Administration On Estrous Synchronisation In Crossbred Cows

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ABSTRACT

Study on synchronisation of estrus was carried out on 48 healthy crossbred animals with two regimens of Dinofertin - 25 mg by I/M route and 10 mg by I/VSM route, at 10 days interval. 75% (12) animals responded to I.M. treatment and 81.75% (13) to I/VSM route. The average time required for onset of estrus in I/M and I/VSM routes was 71.87 ± 1.25 hours and 71.07 ± 1.31 hours with length of synchronised estrus 18.25 ± 0.04 hours and 18.34 ± 0.03 hours, respectively. The number of intense, medium and weak estrus in animals treated with I/M route was 37.5, 35.5 and 25.0 % and in I/VSM 30.76, 44.30 and 26.94 %, respectively. Response to both routes of administration was almost similar.

* * *

The use of PGF_2 alpha for synchronisation of estrus in cattle was first reported by Rowson *et al* (1972) and subsequently confirmed, but its cost is a great constraint. However, its dose can be greatly reduced by changing the route of administration. The alternative routes tried are intrauterine (Shelton, 1973; Moore, 1975) and intravaginal (Ono *et al*, 1982; Singh *et al*, 1987). Present study was initiated to elucidate the efficacy of PGF_2 alpha at reduced dose rate by using intravulvosubmucosal (I/VSM) route of administration and its comparison with the conventional intramuscular (I/M) route.

Materials and Methods

The experimental group comprised of 48 healthy Jersey × Sahiwal crossbred animals : 27 parous, cycling cows 5 to 8 years age and 21 pubertal heifers 2 to 4 years age, maintained at PKV Livestock Instructional Farm, Akola. They were checked regularly for two consecutive cycle activity and those with active corpus

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luteum (CL) were selected for study. They were divided randomly into six groups (Table 1). Dinofertin, (Alved, Pharma. Pvt. Ltd; Madras) 25 mg by I/M and 10 mg I/VSM route was given as double injection regime, 11 days apart, to the experimental animals (Cooper, 1974; Johnson, 1978), which were the closely observed for estrus induction by parading vasectomised bull in the byre at 6 hourly intervals. Besides, they were kept in open paddock with vasectomised bull in the morning hours.

Response of Dinofertin with the two routes was studied on the basis of duration for estrus induction, leugth of estrus and intensity of induced estrus. Total score for each estrus studied was prepared as per Singh and Kharche (1985) with suitable modifications. Heat was classified as intense, intermediate and weak.

Results and Discussion

The synchronisation response to I/M route in groups Ia and IIa was 71.42% and 77.77% and with I/VSM route in groups Ib and IIb, it was 85.71% and 77.77% (Table 1). Twelve out of 16 animals (75%) treated in each group, and 13 (81.75%) from I/M and I/VSM groups, respectively were observed in estrus. The difference was not significant. Similar observations have been reported by Ono *et al* (1982), Holy (1984) and Chauhan *et al* (1986). However, the total response was better than the report of Voh *et al* (1987).

After first and second treatment by I/M route in group Ia and IIa the duration for onset of oestrus was 73.40 ± 1.19 and 70.78 ± 1.95 hours and by I/VSM route in groups Ib, IIb it was 71.33 ± 2.03 and 70.85 ± 1.78 hours. These observations are in agreement with Orihuella *et al* (1983). The average duration of 75.15 ± 1.08 hours required for onset of estrus after first treatment was significantly longer ($P < 0.005$) than that for the second treatment (67.76 ± 1.00 hours). Similar trend of reduced interval with the second treatment is reported by Cooper (1974) and Johnson (1978). However, the overall duration required for onset of estrus with I/M route was 71.87±1.25 hours and with I/VSM route 71.07 ± 1.31 hours.

The heifers treated with both route remained in estrus for shorter period (16.80 ± 0.66 and 17.33 ± 0.35 hours) as compared to cows (19.28 ± 0.38 and 19.21 ± 0.35 hours) with non-significant difference. Likewise, the length of estrus showed no significant difference between treatment (18.30 ± 0.30 hours) and (17.12 ± 0.65 hours) control groups. These findings are comparable with that of Voh *et al* (1987). The length of estrus observed however, was longer as compared to the reports of Galina *et al* (1982), Orihuela *et al* (1983) and Voh *et at* (1987). The average length of oestrus observed in I/M and I/VSM groups was 18.25 ± 0.04 and 18.34 ± 1.035 hours, was not significant. The efficacy with I/VSM route is comparable to that of I/M route.

The animals showing intense, medium and weak oestrus in I/M group was 37.5%, 37.5% and 25% and in I/VSM group 30.76%, 42.30% and 26.94%, respectively. Intensity was almost similar in both the groups which is similar to the findings of Singh *et al* (1987).

It is therefore concluded that the route of PGF_2 alpha administration does not change its efficacy regarding duration, length and intensity of synchronised estrus in corssbred females. Thus, 2 ml (10 mg) of Dinofertin by I/VSM route can be used effectively to bring about luteolysis for estrus synchronisation.

Table 1 : Synchronisation response, duration for onset of oestrus and length of estrus in animals treated with PGF_2 alpha (Dinofertin) by two routes of administration.

Group	Class	Route & Dose	First Treatment				Second Treatment			
			No. of animals		Average Duration (hrs.)	Average length of estrus (hrs.)	No. of animals		Average Duration (hrs.)	Average length of estrus (hrs.)
			Treated	Detected			Treated	Detected		
Ia	Heifer	I/M 5ml (25 mg)	7	5 (71.42%)	76.00 ± 1.67	16.20 ± 0.96	7	5 (71.42%)	70.80 ± 0.48	17.40 ± 0.92
Ib	Heifer	I/VSM 2ml (10 mg)	7	6 (85.71%)	75.66 ± 2.34	16.83 ± 0.79	7	6 (85.71%)	67.00 ± 2.29	17.83 ± 0.70
IIa	Cows	I/M 5ml (25 mg)	9	7 (77.77%)	74.42 ± 2.95	19.00 ± 0.57	9	7 (77.77%)	67.14 ± 1.84	19.57 ± 0.48
IIb	Cows	I/VSM 2ml (25 mg)	9	7 (77.77%)	74.85 ± 1.62	19.00 ± 0.53	9	7 (77.77%)	66.85 ± 2.42	19.42 ± 0.48
IIIa	Heifer	Control	7	7	–	15.71 ± 1.10	–	–	–	–
IIIb	Cows	Control	9	9	–	18.22 ± 0.59	–	–	–	–

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Efficacy Of Gonadotrophin Releasing Hormone (Receptal) Alone And In Combination For Estrus Induction In Anestrus Crossbred Cows

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ABSTRACT

Twenty eight anestrus crossbred cows were divided equally in four groups (three treatment and one control). In addition a normal cycling group of 7 crossbred cows was also taken as a control. For studying estrus response three Receptal (GnRH analogue), single dose therapies were used in anestrus (smooth ovary) crossbred cows in the form of Receptal (5 ml) alone, Receptal (5 ml) primed with Estradiol 17-B (1 mg in 1 ml alcohol) and Receptal (5 ml) pretreated with Tonophosphan (5 ml daily for 3 days) by intramuscular route. In Receptal alone group 100% cows manifested estrus at an average post-treatment interval of 21.00 ± 6.37 days with 57.14% C.R. The corresponding values for Receptal primed with Estradiol and Receptal pretreated with Tonophosphan groups were 85.71%, 12.83 ± 7.18 days, 66.67% and 100%, 23.57 ± 6.29 days and 71.43% respectively. Based on the response obtained Receptal pretreated with Tonophosphan was adjudged as the best therapy, followed by Receptal primed with Estradiol and Receptal alone for the treatment of anestrus with smooth ovaries.

* * *

Anestrus is the most common single cause of infertility in cattle. Administration of GnRH causes dose related increase in the serum concentration of LH and FSH in cattle (Bosu, 1982). This property of GnRH has been used to advantage in the treatment of different fertility disturbances. In the present study an attempt has been made to know the efficacy of GnRH to combat anestrus in crossbred cows.

Materials and Methods

Crossbred cows between 3 to 10 years of age, with smooth ovaries and absence of normal cyclical changes in genitalia and failing to exhibit behavioural signs of heat until 60 days postpartum were selected as experimental animals. Thus 35 selected (7 normal cycling and 28 anestrus) crossbred cows were divided into five groups with 7 animals in each group (two control and three treatment) as detailed below

Group I : Normal cycling control without treatment

Group II : Anestrus control without treatment

Group III : Receptal 5 ml (equivalent to 200 μg GnRH) i/m

Group IV : Estradiol 17-B 1 mg (in 1 ml alcohol) i/m followed by Receptal 5 ml i/m 18 hours later.

Group V : Tonophosphan 5 ml i/m for 3 days followed by Receptal 5 ml i/m 24 hours later.

Following treatment, all the crossbred cows were closely observed, daily morning and

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evening for estrus response and for presence of corpus luteum, on day 10 of treatment. All animals responding to treatment were subjected to AI twice on day of estrus, 10 to 12 hours apart. Similarly, the crossbred cows of control group II were subjected to AI at natural (spontaneous) oestrus. Gynaeco-clinical examination was done on the day of estrus to classify the type of estrus on score card basis with 50 as maximum score (Awasthi and Kharche, 1989). Pregnancy diagnosis to confirm pregnancy was done 60 days of service by gynaeco-clinical examination.

Results and Discussion

Receptal alone (Group III) : All anestrus crossbred cows exhibited presence of C.L. on day 10 of treatment and estrus at day 21.00 ± 6.37. On total estrus intensity basis, 3 cows showed intense, 2 intermediate and 2 weak estrus response. The mean total score during estrus was 37.14 ± 2.63. In 5 out of 7 crossbred cows, estrus was detected by external visual symptoms and in 2 by teaser bull. Out of 7 crossbred cows served, 4 had conceived. These findings are in agreement with the findings of Dhoble and Gupta (1981) who reported mean time to response as 29.17 days. Administration of GnRH causes dose related increase in the serum concentration of LH and FSH in cattle (Bosu, 1982). Anterior pituitary in anestrus animals is found to be rich in gonadotrophic hormone than in cyclic animals, thus suggesting inadequte hypothalamic stimuli for the release of gonadotrophic hormone to act on ovaries and induce estrus.

Receptal primed with Extradiol (Gronp IV) : Six out of 7 crossbred cows (85.71%) responded to treatment and exhibited estrus at an average post-treatment interval of 12.83 ± 7.18 days. On total score basis, one crossbred cow showed intense, 3 intermediate and 2 weak estrus. Mean total score at estrual phase was recorded as 33.83 ± 2.93. Estrus was detected in 4 crossbred cows by external visual symptoms and in 2 by teaser bull. Out of 6 crossbred cows served, 4 had conceived. First ovulation after calving (more than 90 days) was associated with ovarian steroidogenic incompetence (Gyawu *et al.*, 1984). Estradiol acts at two levels (pituitary and hypothalamus) and increases the sensitivity of pituitary gonadotrophin-producing cells to the competent hypothalamic hormone, LHRH and also increases the pituitary glands responsiveness to LHRH.

Receptal Pretreated with Tonophosphan (Group V) : All 7 crossbred cows (100%) of this group exhibited presence of C.L. on day 10 of treatment and estrus on an average post treatment interval of 23.57 ± 6.29 days. On total score basis, 3 cows showed intense, 3 intermediate and one weak estrus response. Mean total score at estrus was 38.29 ± 2.62. Estrus was detected by external visual symptoms in 5 and by teaser bull in 2 crossbred cows. All 7 crossbred cows were served at heat, out of which 4 had conceived. Becze (1964) opined that lack of dietary phosphorus may lead to anestrus. Bhandari *et al* (1975) reported that Tonophosphan might have corrected the deficiency syndrome arising, probably, due to marginal intake of phosphorus to promote gonadal and genital activity.

Normal cycling control (Group I) *and Anoestrus Control* (Group II) : On total score basis, out of 7 crossbred cows of control group I, 4 showed intense, 2 intermediate and 1 weak estrus response. In 4 crossbred cows estrus was detected by external visual symptoms and in

3 by teaser bull. All 7 crossbred cows were subjected to service, but only 4 had conceived. In anestrus control group II, out of 7 cows one cow showed intermediate and one weak estrus response. In one cow estrus was detected by external visual symptoms and in one by teaser bull and only one had conceived.

Thus it may be concluded that Receptal pretreated with Tonophosphan was found to be best therapy, followed by Receptal primed with Estradiol 17-B and Receptal alone and hence is the therapy of choice for combating anestrus in cows.

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Oestrus Synchronization And Fertility In Buffalo Heifers Using Carboprost Tromethamine*

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ABSTRACT

The efficacy of Carboprost Tromethamine (CT) a synthetic analogue of PGF_2 alpha was studied at lower dose levels in oestrus synchronization and fertility in buffalo heifers. Thirty two cycling Surti buffalo heifers were randomly allocated into four groups of eight each. Group I served as control, while II, III and IV received single IM injection of CT on day 10 of natural oestrous cycle. The time taken for onset of oestrus in the CT treated groups was 67.25 ± 3.83, 65.87 ± 3.38 and 57.50 ± 4.22 hr respectively. The conception rates (%) for the four groups were 14.29, 12.50, 25.00 and 62.50, respectively.

* * *

Among all the agents employed for oestrus synchronization, PGF_2 alpha and its synthetic analogues have proved to be the best. It is evident that PGF_2 alpha can bring about

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luteolysis even at lower dose levels (Narayana and Honappa, 1986). Hence, the present study was designed to check the efficacy of carboprost Tromethamine (CT), a synthetic analogue of PGF_2 alpha (Upjohn, U.K.) at lower dose levels in oestrus synchronization and fertility in buffalo heifers.

Materials and Methods

Thirty two regularly cycling Surti buffalo heifers weighing 250 to 350 kgs from the herd of Buffalo Breeding Project, Dharwad were randomly allocated into four groups of eight each and were kept under routine management conditions of the farm. Group I served as control while Group II, III and IV received a single intramuscular (IM) injection of CT at the rate of 25, 50 and 100 μg per animal, respectively on day 10 of their natural oestrous cycle, following confirmation of presence of corpus luteum on one of the ovaries.

Following the CT injection, oestrus was detected by visual observation, examination of cervico-vaginal mucus (CVM) and rectal palpation techniques. The animals in oestrus were inseminated twice with frozen semen at 12 and 18 hr interval and fertility assessed gynaecoclinically 10 weeks following insemination.

The CVM was collected aseptically and subjected for study of arborization pattern and pH using standard techniques. The time taken for onset of oestrus following CT injection and conception rates were recorded. The data were analysed statistically.

Results and Discussion

The mean pH, typical ferning percentage of CVM, mean time taken for exhibition of oestrus following CT injection and conception rates for all the four groups under study are presented in Table 1.

All the animals under the CT treatment groups exhibited oestrus, however the animals of highest dose group (100 μg) had much pronounced oestrus expression which is suggestive of complete luteolysis, wherein marked drop in progesterone level and marked increase in oestradiol 17 β might have occurred. The improved conception rate and mean time taken for estrus exhibition following CT injection in Group IV concur with those of Khurana *et al* (1981) and Chauhan *et al* (1982) employing higher doses of PGF_2 alpha in buffaloes. On the contrary, the poor conception rates recorded in the lower dose groups of 25 and 50 μg of CT may be attributable to incomplete luteolysis.

The animals exhibiting atypical fern pattern failed to conceive, indicating a positive relationship between typical fern pattern and conception. The results of the present study are in confirmity with Luktuke and Roy (1967) and Glotra *et at* (1969).

The pH values of CVM recorded in the present study are in agreement with Narasimhan *et al* (1980). There was a tendency of high conception rate with alkaline pH (7.50 to 8.50) suggesting that alkaline pH may favour better sperm survival in the female genital tract (Pattabiraman *et al*, 1967).

It is concluded that 100 μg of CT can be employed for oestrus synchronization with better fertility in buffaloes, thereby reducing the cost of PGF_2 alpha therapy which is of immense value to the poor farmer.

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Table 1. pH, Arborization pattern of CVM, mean time for onset of oestrus and conception rate in control and CT treated buffalo heifers.

S.No.	Parameters	Groups			
		I	II	III	IV
1.	Mean pH	7.29 ± 0.32	7.06 ± 0.32	7.44 ± 0.26	7.69 ± 0.25
2.	Typical ferning (%)	57.14	37.50	62.50	87.50
3.	Mean time(hr)	7/8 exhibited oestrus	67.25 ± 3.83	65.87 ± 3.38	57.50 ± 4.22
4.	Conception rate (%)	14.29	12.50	25.00	62.50

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Effect Of Season On Superovulation And Embryo Recovery In Sahiwal And Crosses Of Holstein X Sahiwal Donor Cows

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The effect of season upon superovulation and embryo recovery has been studied in *Bos taurus* cattle (Shea, *et al*, 1984; Massy and Oden, 1984). The effect of season upon superovulation and embryo recovery has not been studied well in crosses of *Bos taurus X Bos indicus* and *Bos indicus* cattle. Therefore, the object of this study was to determine seasonal effect on superovulation and embryo recovery in crossbred and pure Sahiwal cows.

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Superovulation was induced in 87 donor animals (crosses of Holstein X Sahiwal n = 74 and pure Sahiwal n = 13) during a 3–year period (1986 to 1989) at New Delhi. Superovulation was induced from day 9 to 12 of estrus cycle with I. M. injection of 28 mg FSH-P twice a day for four days in descending doses. PGF_2 alpha was given I.M. 48 hour after initiation of superovulatory treatment. All the animals were inseminated with freshly ejaculated semen at 0, 12 and 24 h. after estrus detection.

Embryos were collected 7 d after the last insemination as per Totey *et al* (1988). The seasons were defined thus (i) Summer : March to June, (ii) Rainyseason : July to October and (iii) Winter : November to February.Data was evaluated by analysis of variance (ANOVA).

Out of total 74 crossbred donor animals treated for superovulation, 73.0% responded to treatment. Maximum (81.0%) donors responded during winter season (17/21), followed by summer 70.3% (19/27) and lowest during rainy season 69.2% (18/26). However, difference is not significant, whereas 75% Sahiwal donors responded to treatment during rainy season and lowest (20%) during winter season.

Mean (± SE) ovulations were more during rainy season (8.7 ± 1.4) than winter (6.9 ± 0.8) and summer (6.1 ± 0.5) in crossbred cows ($p > 0.05$). Likewise these values were more in Sahiwal cows during rainy season (11.3 ± 1.7) than in winter (7.0 ± 0.0). Superovulatory response (11.3 ± 1.7) during rainy season was significantly more ($P < 0.05$) in Sahiwal cows than crossbred donor cows (8.7 ± 1.4). Moreover, overall superovulatory response was better in Sahiwal than crossbred cows. It is not clear why this difference exists. It may be due to lower levels of circulatory gonadotropins in *Bos indicus* cows, rather than lower ovarian responsiveness (Griffin and Randel, 1978). This may explain why the ovaries of *Bos indicus* are more responsive to exogenous gonadotropins than those of *Bos taurus* cows (Munro, 1986).

Embryo recovery rate was more during rainy season in crossbred (5.9 ± 0.7) and Sahiwal (8.5 ± 1.8) cows, than in summer and winter seasons (Table 1). Maximum recovery of embryos occurred during October in all donor cows.

Number of transferable embryos were more during rainy season in crossbred (2.4 ± 0.8) and Sahiwal cows (4.5 ± 1.4) than in summer and winter seasons (Table 1). However, the difference was not significant ($P > 0.05$). There was a trend towards low donor performance in terms of superovulatory response, embryo recovery and transferable embryos during winter season in both the breeds. This may be due to lower preovulatory LH surge at estrus during winter season (Randel, 1986). Overall data shows that seasons, month and year did not significantly affect total ovulations, total embryo recovery and transferable embryos ($P > 0.05$). Our results are in agreement with Massey and Oden (1984); Randel (1984) and Shea *et al*, (1984). However, environmental factor such as nutrition, light conditions, temperature, photoperiodic variations and genetic differences must be studied thoroughly. Though there is no significant effect of season on superovulation and embryo recovery, both the crossbred and Sahiwal cows

showed better performance during July-October than other seasons of the year.

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Table 1 : Effect of seasons on ovulations, total embryo recovery and transferable embryo recovery in cross-bred and Sahiwal donor cows.

Seasons	No. of Donors Treated	No. of Donors Responded (%)	Mean Ovulations	Mean Total Embryos	Mean Transferable Embryos
Crossbred					
Winter	21	17 (80.9)	6.94 ± 0.84	3.64 ± 0.62	1.47 ± 0.39
Summer	27	19(70.3)	6.10 ± 0.59	3.84 ± 0.50	2.52 ± 0.49
Rainy	26	18 (69.2)	8.77 ± 1.42	5.94 ± 0.95	2.44 ± 0.83
Sahiwal					
Winter	5	1 (20)	7.0 ± 00	2.0 ± 00	00 ± 00
Rainy	8	6 (75)	11.33 ± 1.73	8.5 ± 1.88	4.5 ± 1.48

Effect of summer could not be evaluated because of limited number of cows available/treatment.

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Ovarian Changes In Black Bengal Goat During Various Phases Of Reproduction*

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ABSTRACT

Ovarian changes were studied on Day 12 post-ovulation in non- pregnant, normal cyclic and pregnant goats on Day 30, 90 and 122 of gestation. Small sized multiple follicles were common in the phases studied. CL protruded over ovarian surface except on Day 12 post-ovulation, wherein few CL were deeply embedded in stroma. CL colour varied from light to deep pink. The size and weight of ovaries with CL verum increased gradually with progressive increase in the size of CL and advancing gestation period vis-a-vis Day 12 post-ovulation period.

* * *

It is an established fact that ovarian changes are related to the reproductive efficiency of animals. Systematic changes in ovaries of Black Bengal Goat after ovulation and during different stages of pregnancy have not been studied as revealed from available literature. The present study was therefore undertaken.

Materials and Methods

Six pre-pubertal Black Bengal goats *(Capra hircus)* of uniform age and weight reared under identical conditions of housing, care and management were included in the present study. The goats on attainment of puberty at the age of 8 to 9 months were not served during the initial three estruses. The length of estrum was calculated by observing three successive estrus periods at an interval of 20 to 21 days as per Harison (1948) and Roberts (1971), which was found to vary from 36 to 48 hours. 3 goats, out of 6 were covered during the 4th estrum by healthy and sexually mature buck. The remaining 3 goats in estrus were separated from the flock to prevent mating. They were used to study the extent of development of normal cyclic CL on Day 12 post-ovulation. Ovulation time was considered to occur by 40th hour on an average after the onset of estrus as per Harison (1948). 3 goats which did not come to estrus after mating became pregnant. Ovary containing CL of these pregnant goats, each at Day 30, 90 and 122 of gestation were studied.

The ovaries were collected by usual surgical technique on mid-ventral approach under lumbo-sacral segmental spinal anaesthesia (Klide, 1971) as well as local linear block following all pre and post-surgical cares. Ovaries were separated from extraneous tissues and various parameters such as shape, size, visible structures and colour of CL were studied as quickly as possible in an air conditioned laboratory to avoid shrinkage and colour change. The length (L), width (W) and thickness (T) of the ovaries were measured as per Singh *et al* (1974). The ovaries were then halved length-wise and right through the middle and

* Part of the Thesis submitted by Senior author for the award of Ph.D. Degree to BCKV, Mohanpur, 1987.

point of protrusion of CL when present, and the greatest length and breadth of the CL were measured as per Luktuke and Rao (1962). The diameter of the knob-like protrusion of the CL over the ovarian surface was also measured. The presence of superficially visible ovarian follicle number and size was also recorded.

Results and Discussion

The shape of ovaries was mostly oval, except a few in which it was roundish, elongated and tapering. Harison (1948) and Arthur *et al* (1982) reported bean and nearly spherical ovarian shape in goat. The slight differences obtained in the present study might be due to breed differences. The ovaries of goats on Day 12 after ovulation in non-pregnant, normal cyclic condition and on Day 30, 90 and 122 of gestation showed presence of small sized (1-2 mm) multiple follicles, which is in full agreement with the findings of Harison (1948) who stated that it may be an expression of growth stimuli which begins in the embryo and continues during reproductive life of the goat or due to variable proportion of germ cells, epithelium and ovarian stroma in different species.

At Day 12 post-ovulation in non-pregnant cyclic goats, 2 out of 4 ovaries with CL showed fully developed protruding CL while in other 2, it was found embedded in the ovarian stroma. At Day 30 of pregnancy, protrusion of CL was 4 mm in diameter and 2 mm in height. During Day 90 and 122, the protruded CL were 5 mm and 6 mm in diameter and 2 mm and 3 mm in height respectively. These findings being first of its type, could not be compared due to paucity of specific information.

The colour of CL in non-pregnant, normal cyclic goats at Day 12 post-ovulation and in pregnant goats at different stages of pregnancy, was found to be varying from light pink to deep pink. This observation is allied to that of Arthur *et al* (1982) in goat. The light to deep pink colour of CL of goats in different conditions studied, might be an indication of increased vascularity at maximal developmental and functional state of the gland.

The length and width of ovaries with CL at Day 12 post-ovulation in non-pregnant, normal cyclic goats was found to vary from 1.35 to 1.60 cm. (average 1.46 ± 0.05 cm.) and from 0.70 to 0.85 cm. (average 0.80 ± 0.03 cm.) respectively (Tables 1, 2). The thickness ranged from 0.95 to 1.20 cm. (average 1.05 ± 0.05 cm.). The present findings were within the ranges reported by Rahaman *et al* (1977). The length of the CL in the ovary at Day 12 post-ovulation varied from 1.00 to 1.10 cm. (average 1.05 ± 0.02 cm.) and breadth ranged from 0.90 cm. to 1.00 cm. (average 0.95 ± 0.02 cm.). These findings were in close agreement with Harison (1948) who reported the diameter of CL in non-pregnant goat at Day 12 after ovulation as 1.10 cm. The size of ovary with CL at Day 30, 90 and 122 of pregnancy in the present study was L 1.50 × W 0.87 × T 1.10 cm; L 1.57 × W 0.90 × T 1,20 cm. and L 1.70 × W 1.00 × T 1.41 cm; while the length and breadth of CL verum during these three gestation periods were 1.10 × 1.00 cm; 1.20 × 1.10 cm. and 1.30 × 1.20 cm., respectively. The average weight of ovary with fully developed CL in non-pregnant state at Day 12 post-ovulation, at Day 30, 90 and 122 of pregnancy was recorded as 1.36 ± 0.02 gms, 1.49 gm, 1.52 gm and 1.57 gm. respectively. These findings could not be compared due to lack of similar specific work. However, they clearly indicate that the size and weight of ovary with CL in pregnant goat increased gradually with

advancing pregnancy due to progressive increase in the size of CL which was larger than that obtained at Day 12 after ovulation of normal estrus cycle. This may continue until parturition, as reported in the cow by Hafez (1980).

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Table 1 : Average weight and size of ovaries with CL in non-pregnant and pregnant goats.

Stages of Ovary with CL	Number of observations	Average weight ± S.E. of ovary with CL (gm)	Average size with ±S.E. of ovary with CL (cm)		
			Length	Width	Thickness
12th day after ovulation in non-pregnant condition	4	1.36 ± 0.02 (1.31 – 1.42)	1.46 ± 0.05 (1.35 – 1.60)	0.80 ± 0.03 (0.70 – 0.85)	1.05 ± 0.05 (0.95 – 1.20)
30th day of pregnancy	1	1.49	1.50	0.87	.10
90th day of pregnancy	1	1.52	1.57	0.90	1.20
122nd day of pregnancy	1	1.57	1.70	1.00	1.41

CL = Corpus luteum Figures in the parenthesis indicate range.

Table 2 : Average size of the Corpus luteum in non-pregnant and pregnant goats.

Stages of CL	Number of observations	Length (cm)	Breadth (cm)
12th day after ovulation in non-pregnant condition	4	1.05 ± 0.02 (1.00 – 1.10)	0.95 ± 0.02 (0.90 – 1.00)
30th day of pregnancy	1	1.10	1.00
90th day of pregnancy	1	1.20	1.10
122nd day of pregnancy	1	1.30	1.20

Figures in the parenthesis indicate range.

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Studies On Synchronization Of Oestrus In Does*

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ABSTRACT

Out of 20 does treated with Lutocycline, 17 (85 %) exhibited oestrus in 80.52 ± 3.04 hours with average duration of 24.94 ± 0.78 hours.

* * *

Synchronization of oestrus has been defined as the regulation of oestrus cycle at will. The control and synchronization of oestrus in does has become a subject of major interest in recent years particularly in embryo transfer technology. Synchronization of oestrus of the donor with that of recipient is necessary for successful embryo transfer. For optimum results the recipient should be in oestrus within ± 12 hours of the donor. Pregnancy rates decline drastically if the difference is greater than 48 hours, since the uterine environment of the recipient may not be favourable for implantation and survival of the embryo (Hafez, 1985). Oestrus in does can be synchronized with progesterone injections (Inj. Lutocycline), intravaginal sponges (Medroxyprogesterone acetate), by oral administration (Melengesterol acetate), by injecting $PGF_2 \propto$ or by subcut ear implants. The present study was undertaken to assess the efficacy of Inj. Lutocycline for synchronization of oestrus. By exposing the animals to progesterone treatment for a prolonged period conditions simulating to those of normal cycling corpus luteum are achieved. Thus as a withdrawal effect of progesterone there is stimulus for the follicular growth and animal may come in heat.

Materials and Methods

A total of 20 Osmanabadi does and their crosses with Alpine, Beetal and Saanen, aged 3 to 5 years and weighing 22 kg to 38 kg were slected. The does were healthy, normal cyclic with good reproductive efficiency, having earlier 2-3 normal kiddings and history of last kidding at least 2 months earlier. They were kept under identical feeding and managemental practices and divided in 4 groups comprising 3, 3, 8 and 6 does in each group. The does from these groups were treated with Inj. Lutocycline (Hindustan CIBA-GEIGY limited, Bombay 440 020). 0.5 ml (12.5 mg progesterone) I.M. per doe per day for 17, 16, 15 and 14 days respectively.

The does were closely observed for exhibition of oestrus by parading a vasectomized buck daily at 7 a.m. and 4 p.m. and also by visual observations throughout the study period. Duration of oestrus was determined as the period between first and last mounting by the vasectomized buck.

Results and discussion

Out of three recipients from group I, II (66.67%) exhibited oestrus 87.5 ± 8.5 hrs after last progesterone injection with an average duration of oestrus of 27.0 ± 3.0 hrs. Out of

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three does from group II, only one (33.34%) doe exhibited oestrus after 96 hrs of last injection with duration of oestrus 24 hrs. In group III, all 8 (100%) does exhibited oestrus after 82.62 ± 4.75 hrs of last injection with average duration of oestrus 25.75 ± 1.38 hrs. In the last groups, all six (100%) does exhibited oestrus 75.8 ± 2.45 hrs after last progesterone injection and the duration of oestrus was 23.2 ± 0.8 hrs.

The overall synchronization of oestrus was 85% (17/20), with duration of oestrus 80.52 ± 3.04 hrs and length of oestrus 24.94 ± 0.78 hrs.

The present finding regarding synchronization (%) in does following progesterone treatment is in agreement with that (84.62%) reported by Patil *et al*, (1984). The findings are significantly lower than 100% and 96.12% as reported by Kiessling *et al* (1986) and Agrawal (1987) respectively.

The findings reported for exhibition of synchronized oestrus are in agreement with those reported by Godd and Tervit (1984), Patil *et al* (1984), and Greyling *et al*. (1985) as 3.5 days, 87.62 ± 4.53 hours and 81.0 ± 17.0 hours for 14 days treatment respectively. However these are significantly higher than those reported by Bongso *et al* (1982), Bretzlaff and Madrid (1985), Greyling *et al* (1985) and Kiessling *et al* (1986) as 16 to 40 hours, 24 hours, 74 ± 18 hours for 16 days treatment and 52 ± 15 hours for 18 days treatment and 48 hours respectively. The findings are significantly lower than those (4 days) reported by Agrawal (1987).

Table 1 : Exhibition and duration of synchronized oestrus in does (in hours)

Group	Goat No.	Breed	Body Weight (kg)	Age Y.	Age M.	Progesterone treatment (I. M. route)	Exhibition of oestrus (hrs)	Duration of oestrus (hrs)
I	G-09	Alpine cross	36	3	10	Inj. Lutocycline	–	–
	G-14	Alpine cross	32	5	8	0.5 ml per day per doe	79	30
	G-36	Saanen cross	23	5	4	for 17 days	96	24
							Av 87.5 ± 8.5	Av 27.0 ± 3.0
II	G-17	Osmanabadi	26	4	6	Inj. Lutocycline	–	–
	G-29	Osmanabadi	34	5	3	0.5 ml per day	96	24
	G-34	Alpine cross	35	4	2	per doe for 16 days	–	–
III	G-37	Osmanabadi	25	3	5	Inj. Lutocycline	96	24
	G-26	Alpine cross	31	5	3	0.5 ml per day	81	24
	G-30	Osmanabadi	29	6	7	per doe for 15 days	96	28
	G-27	Alpine cross	24	4	2		81	32
	G-38	Osmanabadi	27	3	6		58	24
	G-39	Alpine cross	22	5	4		72	30
	G-48	Alpine cross	26	3	1		96	20
	G-49	Osmanabadi	30	3	9		81	24
							Av 82.62± 4.75	Av 25.75± 1.38
IV	G-44	Beetal cross	38	4	3	Inj. Lutocycline	72	20
	G-02	Osmanabadi	28	6	5	0.5 ml per day	84	24
	G-01	Alpine cross	30	33	11	per doe for 14 days	72	24
	G-40	Osmanabadi	27	5	9		79	24
	G-45	Beetal cross	24	4	3		58	24
	G-46	Osmanabadi	26	4	2		72	24
							Av 72.83± 3.58	Av 23.34± 0.66
						Overall	80.52 ± 3.04	24.94 ± 0.78

The duration of oestrus after synchronizatiom reported in present study is in the normal prescribed range of 20 to 30 hours (Sane *et al.* 1982)

The reasons for significantly higher or lower values (%) for exhibition of synchronized oestrus may be due to breed, nutritional status and hormonal profile variations in animals studied.

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Induction And Synchronization Of Oestrus In Anoestrous (Acyclic) Recipient Goats

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ABSTRACT

Thirty eight parous acyclic goats (11 Non-descript and 27 Jakhrana) were subjected randomly to three treatment regimes for induction of oestrus. Animals that exhibited oestrus within 96 h post treatment were 8(66.66%), 5(62.50%), and 4(22.22%) in treatment I, II and III respectively. The duration of induced oestrus was 36.00 ± 6.36 h (range : 12-60 h), 45.60 ± 2.14 h (range : 36-48 h) and 36.00 ± 10.39 h (range : 24-72 h) in treatment I, II and III respectively (Table 1). Difference in oestrus response was statistically non-significant ($P > 0.05$). The animals treated with prostaglandin analogue alone exhibited very poor response (22.22%) with significantly lower

($P < 0.01$) value than other two treatment groups probably due to the acyclicity (absence of corpus luteum) of goats, a stage mostly observed in summer season.

* * *

Close synchronization of oestrus in donor and recipient animals is considered an important facet for the success of embryo transfer programmes in a number of species (Moore and Shelton, 1964; Rowson and Moor, 1966). Few informations on synchronization of oestrus/ovulation in cyclic goats are available (Serna *et al* 1978; Moore and Eppleston, 1979; Pendleton *et al* 1986; Bondurant, 1986; Corteel, 1975; Agrawal, 1987). The author (KPA) in his 20 years experience to work on different goat breeds has observed a trend of seasonal anoestrous (acyclicity) in summer months (March to June). The objective of the present experiment was to study the effect of Veramix Vaginal sponges as source of progestagen and Carboprost tromethamine, a prostaglandin analogue separately or in combination, on induction/synchronization of oestrus in anoestrous (acyclic) goats used as recipients for embryo transfer programme being carried out at Goat Reproduction and Physiology Division of the Institute.

Materials and Methods

Thirty eight parous acyclic goats (11 Nondescript and 27 Jakhrana) were subjected randomly to three treatment regimes for induction of oestrus in summer (May-June 1989). Treatment I (12 animals) : Vermix Vaginal Sponge (60 mg medroxy progesterone acetate Upjohn, U.K.) was placed in situ for 18 days. Treatment II (8 animals) : 62.50 μg of prostaglandin analogue (Carboprost tremethamine, Upjohn, U.K.) was injected IM 24 h before withdrawal of Veramix Vaginal Sponge. Treatment III (18 animals) : 125 μg of prostaglandin analogue in equally divided two doses was injected IM, 11 days apart. After withdrawal of treatment, the goats were detected for oestrus at 12 h interval by using an aproned buck. Normal deviate and 't' tests were applied to interpret the results.

Results and Discussion

The number of animals exhibited oestrus within 96 h of post treatment were 8 (66.66%), 5(62.50%) and 4(22.22%) in treatment I, II and III respectively. The duration of induced oestrus was 36.00 ± 6.36 h (range : 12-60 h), 45.60 ± 2.14 h (range 36-48 h) and 36.00 ± 10.39 h (range 24 - 72 h) in treatment I, II and III respectively (Table 1). Difference in oestrus response in treatment I and II was statistically insignificant ($P > 0.05$), indicating that administration of prostaglandin analogue 24 h before withdrawal of Veramix Vaginal sponge did not have any additional benefit over Veramix vaginal sponge when used alone. The animals treated with prostaglandin analogue alone (treatment III) exhibited poor response (22.22%) and the values were significantly lower ($P < 0.01$) than other two treatments (I and II). Poor response of treatment III (Prostaglandin analogue double dose schedule) appears due to the acyclicity (absence of corpus luteum) of goats. On the basis of findings in the present experiment, it can be concluded that prostaglandin analogues are not effective in inducing oestrus in acyclic goats, a stage mostly observed in summer season.

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Table 1 : Effect of Different Treatments on Induction of Oestrus in goats.

Treatments	No. of Animals treated	Animals exhibited oestrus within 96 h of post treatment No. (%)	Oestrus duration (Hour)
I	12	8 (66.66)^a	36.00 ± 6.36^a
II	8	5 (62.50)^a	45.60 ± 2.14^a
III	18	4 (22.22)^b	36.00 ± 10.39^a

Values with different superscripts differ significantly ($P < 0.01$)

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Superovulatory Response To Two Hormonal Regimens In Non-descript Goats

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ABSTRACT

Twelve goats were superovulated, with 1000 IU of pregnant mare serum gonadotrophin (PMSG) followed by 600 IU the following day. Half of the goats were primed with progesterone I.M. @ 12.5 mg per day prior to PMSG administration. The other half were given prostaglandin F_2 alpha (PG) IM @ 7.5 mg per animal with second IV dose of PMSG. Human Chorionic Gonadotrophin (HCG) 1500 I.U. was administered IM next day on appearance of oestrus in all the animals. No significant differences were observed ($P < 0.05$) between the mean ovulation rate, percentage of ovulation, number of ova recovered, percentage of ova recovery, number of embryos

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recovered and percentage of fertilization of recovered ova in both the groups.

* * *

PMSG and FSH in different dose levels have long been used to induce superovulation in goats (Nishikawa *et al*, 1963; Elsden *et al*, 1978). Progesterone (Ahmed and Maurya, 1981; Indramani and Vadnere, 1989) and prostaglandins (Jain and Madan,1986; Pandiya and Rathore, 1986) to prime the animals and to synchronize oestrus before administration of gonadotrophins for superovulation. The present study was carried out to evaluate the superovulatory response using higher doses of PMSG in non-descript goats primed with progesterone or $PGF_2 \alpha$.

Materials and Methods

Twelve nondescript adult cycling goats of 2-4 years age were divided randomly into two groups of 6 animals each. Animals in group I were given 12.5 mg progesterone (Lyophilic Labs., Bombay) IM injection daily for 14 days. 24 hours after the withdrawal of progesterone therapy, 1000 IU of PMSG (Folligon, Intervet, Holland) was injected IM in each animal followed by 600 IU 24 hours later. Group II animals were also given same doses of PMSG in the luteal phase of oestrus cycle followed by an IM injection of 7.5 mg of PGF_2 alpha (Dinofertin, Alved Lab., Madras) alongwith the second dose of PMSG. 1500 IU of HCG (Chorulon, Intervet, Holland) was given IV in both the groups the next day on appearance of oestrus. The animals were mated with bucks of proven fertility, twice at an interval of 8-12 hours. The animals were subjected to laparotomy 3-5 days after the onset of oestrus for evaluating the superovulatory response and collecting ova.

Results and Discussion

All the goats exhibited oestrus 1 day after the second injection of PMSG. No significant differences ($P < 0.05$) were observed between the mean ovulation rate (16.33 *vs* 11.33), percentage of ovulation (81.67 *vs* 77.27), number of ova recovered (9.67 *vs* 8.0), percentage ova recovery (59.18 *vs* 70.59), number of embryos recovered (7.0 *vs* 6.33) and the percentage of fertilization of recovered ova (72.14 *vs* 75.47) in both the groups.

The higher ovulation rate (16.33) and percentage of ovulation (81.67) as compared to those reported by other workers (Nishikawa *et al*, 1963; Ahmad and Maurya, 1981; Indra Mani and Vadnere, 1989) might be due to higher doses of gonadotrophins used in the present study.

The average number of ova and embryos recovered (9.67 and 7.00) were identical to the reports of Ahmed and Maurya (1981). However, the percentage of ova recovered (59.18%) and the percentage of fertilization (72.41%) were lower than those observed (77.41 and 81.87% respectively) by the above workers. The lower percentages of these two attributes may be due to the higher ovulation rate in this experiment (Shea *et al*, 1976 and Betteridge, 1977).

There was no significant difference between the superovulatory response of the two regimens. The higher percentage of ova recovered and the higher fertilization rate in the PG treated group vis-a-vis progesterone treated group was probably due to lower ovulation rate (11.33) in the former group supporting the findings of Shea *et al* (1976) and Betteridge (1977).

Treatment with gonadotrophins and PG appeared better than the progesterone and gonadotrophin combination for superovulation in normal cyclic goats due to the short duration of treatment and higher ova recovery and fertilization rates.

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A Note On Serum Biochemical Constituents In Cycling And Post Partum Anoestrous Ewes

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Blood serum constituents such as calcium, inorganic phosphorus and total protein are known to play important role in the reporduction of animals. Though this aspect has been investigated in normal cycling ewes, there is very little information on their role in anoestrous ewes (Purohit *et al.* 1986). The present investigation was undertaken to explore the possible relationship of some serum constituents to anoestrum in ewes.

Blood serum of both post partum anestrum and cycling ewes was collected and kept in dry sterile vials. It was analysed for calcium by Clark Collip modification of Kramer-Tisdall method as per Oser (1965). Inorganic phosphorus was estimated as per technique of Spectronic 20 manual. Similarly, serum total protein was estimated by Biuret assay method laid down in the manual. The data was statistically analysed as per Snedecor and Cochran (1967).

The quantitatively estimated values of total protein, calcium, inorganic phosphorus and calcium phosphorus ratio in the blood

serum of post-partum anoestrous and cycling ewes alongwith their Fisher 't' values, are presented in Table 1. The deficiency of protein could affect reproduction in ewes (Purohit *et al.*, 1986 and Rao *et al*, 1988). The significantly lower protein ($P < 0.01$) in the post-partum anoestrum ewes in comparison to cycling ewes presently studied, agrees with the findings of previous workers. Significantly lower calcium ($P < 0.01$) level among anoestrum ewes in comparison to that of cycling ones, is in agreement with the findings of Purohit *et al* (1986). During post- partum period, there is additional requirement of calcium, because of lactation and nursing of lambs. However, the difference in the level of inorganic phosphorus was not statistically significant. Rather, it remained unaltered. The significantly lowered calcium and unaltered inorganic phosphorus is suggestive of disturbed calcium phosphorus ratio.

The decrease in calcium level might be due to lactation stress and pre-natal foetal development and is not compensated during post-partum period due to inadequate feeding of concentrates. However, the depletion of inorganic phosphorus during pregnancy and lactation is adequately compensated by grazing as the grass and forage provide a rich source of phosphorus than calcium.

It is therefore suggested that for maintenance of optimum reproduction, supplementation of calcium and protein diet is essential for ewes maintained under traditional grazing.

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Table 1 : Mean values with S. E. of Blood Serum constituents in anoestrous and cycling ewes ('t' test)

Groups	Total Protein gm / 100 ml	Calcium mg / 100 ml	Inorganic Phosphorus mg / 100 ml	Calcium : Phosphorus Ratio (Ca : P)
Post-partum anoestrous (n = 20)	4.73 ± 0.28 (3.85 – 6.95)	6.16 ± 0.19 (3.20 – 8.20)	6.55 ± 0.23 (5.00 – 7.50)	0.94
Cycling ewes (n = 22)	6.05 ± 0.24 (4.39 – 8.50)	10.76 ± 0.22 (8.90 – 12.50)	5.96 ± 0.24 (4.25 – 8.75)	1.80
't' values comparison between anoestrous and cycling groups	3.26^{**}	2.79^{**}	1.47^{NS}	–

n = number of animals. Figures in parentheses in columns, 2, 3 and 4 indicate range values.

** – $P < 0.01$ NS – Non significant.

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Incidence Of Leptospirosis In Aborting And Repeat Breeding Cows and Buffaloes

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Leptospirosis as an important cause of abortion in cattle, has been reported by various workers (Adinarayanan *et al*, 1960; Knott and Dadswell, 1970; De *et al*, 1983). The present report deals with the serological study undertaken on samples collected from aborting and repeat breeding cows and buffaloes.

Materials and Methods

Sera samples from cows and buffaloes with the history of abortion or repeat breeding were received from Abortion control centre for Cattle, Erode, Tamil Nadu. Samples were tested by microscopic agglutination test (MAT) using four-day-old live cultures of the leptospiral serovars *autumnalis, ballum, bataviae, canicola, grippotyphosa, hebdomadis, icterohaemorrhagiae, pomona* and *pyrogenes* as suggested by Faine (1982). Sera samples which gave a titre of 160 and above were considered positive.

Results and Discussion

Of the 85 sera samples tested, 20 (23.52%) had leptospiral antibody titres ranging from 160-1280 (Table 1). Aborted cows had a higher incidence of leptospirosis (38.46%) compared to 16.66% among repeat breeders, whereas prevalence of leptospiral antibodies in the aborting and repeat breeding buffaloes was almost equal. Serovar *pomona* predominated in aborted as well as repeat breeders. Leptospiral abortions occurred during 5th to 7th month of gestation in cows and buffaloes.

Many workers recorded antibodies to serovar *pomona* in aborted cows (Somasundara Rao and Surendran, 1970; Knott and Dadswell, 1970). Ellis *et al* (1982) reported on *hardjo* abortions throughout gestation and causing early embryonic death as well.

In the present study, high titres of 1280 were recorded indicative of recent infection. Cattle and buffaloes did not show significant differences in the prevalence of leptospiral agglutinins. The study proved the involvement of serovar *pomona* in causing abortion and infertility problems in cattle and buffaloes in Periyar district, Tamil Nadu.

Table 1 : Prevalence of leptospiral antibodies in cattle and buffaloes.

S. No.	Species	Total Tested		Total	Lepto positive in				Total	
		Aborting	Repeater	(n)	Aborting	%	Repeater	%	(n)	(%)
1.	Cow	26	18	44	10	38.46	3	16.66	13	29.54
2.	Buffalo	23	18	41	4	17.39	3	16.66	7	17.07
	Total	49	36	85	14	28.57	6	16.66	20	23.52

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Clinical Trials Of Amikacin On Genital Bacteria Of Repeat Breeding Buffaloes*

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The continuing clinical problem of genital bacterial infections has been paralleled by decrease in the sensitivity of the pathogens to various antibacterial agents. Hence, study was undertaken to assess the effectiveness of Amikacin, a semisynthetic derivative of Kanamycin, on the genital bacteria in vivo as well as *in vitro*.

Cervical mucus samples were collected aseptically, from 22 repeat breeding Surti buffaloes by rectovaginal technique (Panangala *et al*, 1978), for quantitative and qualitative study of bacteria. 2 tablets of Amikacin, each of 1.3 gm were inserted in the uterus for 3 consecutive days and on 4th day, cervical mucus samples were collected again for quantitative study.

Bacterial count was estimated by Standard Plate Count (SPC) method, mixing SPC agar medium with tenfold dilutions of mucus in duplicate. Average colony count from 2 plates was multiplied by dilution factors so as to arrive at the total count per ml of mucus. Isolation of bacteria was done as per Cruickshank *et al* (1975). Each of the isolate was further identified as per Cowan and Steel (1970). The *in vitro* antibiotic sensitivity test was carried out as per Bauer *et al* (1966).

The bacterial count ranged from 2,280 to 1,16,000 per ml of mucus (Av 37,531) before treatment, whereas it ranged from 0 to 4,600 (Av. 1,054) after treatment. This decrease in the count consequent to Amikacin treatment, was found statistically significant ($p < 0.01$) by paired t - test.

Out of the total 30 isolates, predominant organisms were *Diphtheroids, Bacillus* spp.

* Part of M. V. Sc. Thesis submitted by first author.

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and *Micrococcus* spp. Antibiogram of these isolates revealed that Amikacin was most effective (80%), followed by Chloramphenicol (76.67%), Gentamycin (70%), Co-trimexazole (50%), Tetracycline (40%), Penicillin (23.33%), Furazolidone (20%) and Streptomycin (16.66%). Drasar *et al* (1976) also reported effectiveness of amikacin on various gram negative bacteria. Out of the total 26 isolates of gram positive bacteria, 23 and 18 were sensitive to amikacin and gentamycin respectively. Thus, amikacin was found more effective on gram positive bacteria. Amikacin has been reported to be effective against *Staph. aureus* and *Staph. epidermidis* (Sabath *et al*, 1976).

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The Clinical Outcome Of Different Regimes Of Treatment Of Uterine Torsion In Buffaloes

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ABSTRACT

Sixty eight full term pregnant buffaloes with uterine torsion were subjected to rolling on their back (fresh cases) or to caesarean section (delayed/complicated cases) for detorsion. 80% rolled animals were successfully detorted. Those with fully dilated cervix at detorsion had maximum survival rate (95%). The survival rate was minimum (45%) where caesarean followed unsuccessful rolling. Rolling of dam is recommended as treatment of choice in fresh cases and caesarean section when absolutely necessary.

* * *

Torsion of uterus is frequently occurring serious cause of dystocia leading to death of fetus, dam or both, in cattle (Arthur, 1975) and buffaloes (Vashishta, 1983). Rolling of dam with or without abdominal pressure, per-vaginal correction of fetus and caesarian section are among the few treatments in cattle

(Roberts, 1971). Verma *et al* (1974) successfully treated uterine torsion in buffaloes by caesarean section. Pattabiraman *et al* (1979) observed rolling, the non-surgical method as a treatment of choice for torsion in buffaloes. Scanty literature is available on comparative success of these methods for relieving bovine uterine torsion. Present paper deals with comparative studies on success of various treatments for uterine torsion in buffaloes.

Materials and Methods

Seventy three of the buffaloes presented for dystokia in one year to PAU Veterinary Clinics had uterine torsion of various degrees, directions and sites. Depending upon the general health status, chronicity, the type of handling and damage caused prior to presenting, the cases were selected thus :

Group I : Comprised of relatively fresh cases with a recent history of the signs of the disease. These animals were either standing or were able to stand and apparently free from vaginal tears or adhesions of uterus. Each of these animals was rolled maximum upto 4 times on the back in the direction of torsion with a plank pressing on its abdomen (Pattabiraman *et al*, 1979) for detorsion of uterus. Animals having fully dilated cervix following detorsion were delivered soon after. Cases with closed or partially dilated cervix were treated as per need with calcium, fluids, and oxytocin to tone up the uterus, and help in complete dilatation of cervix within the next 12 hrs.

Group II : Cases of torsion > 3 days, obvious utero-omental adhesions, vaginal tears and prostrate cases were subjected to caesarean section performed at precrural site, parallel to the milk vein, for removal of the fetus (Verma. *et al*, 1974).

Group III : Some of the cases from group I, which could not be detorted by rolling or in which the cervix failed to dilate, were subjected to caesarean section.

Results

Out of 177 cases of dystocia handled, 77(66 %) had uterine torsion, all of which had been handled by local quacks or vets earlier. Five cases died before giving any treatment and 4 taken away untreated by the farmers. Out of 55 cases in group I, 44 (80 %) were successfully detorted by rolling. Cervix was fully dilated in 19, partially dilated in 21 and completely closed in 4 cases. Cases with fully dilated cervix were delivered soon after detorsion. One animal died of shock after delivery.

The partially dilated cervix in 19 out of 21 buffaloes, dilated completely and delivered within 12 hrs after rolling. In this subgroup, 2 buffaloes died undelivered and 3 died after delivery. Out of 4 buffaloes with closed cervix, one delivered within 12 hrs due to spontaneous dilatation of cervix.

Two buffaloes between 7-8 months of gestation carrying live fetus and closed cervix, were discharged after detorsion, hence deleted from calculations due to lack of follow-up information.

The improvement by rolling the dam, if any, was evident after the first or second roll. In cases where 2 rolls failed to show any improvement, further rolling had no beneficial effect. Rolling failed to detort 11 (20 %) of 55 cases of group I. Two buffaloes were taken away untreated, 3 died following rolling and 6 were subjected to caesarean section, 2 of which died following surgery.

In 13 cases found unfit for rolling, caesarean section was performed (group II).

Five cases died during or after operation and 8 cases discharged after complete recovery within 10-15 days.

Eleven buffaloes of group I, which failed to respond, were subjected to caesarean section (Group III), of which 5 (45 %) survived with complete recovery. Within group III, the caesareans performed soon after unsuccessful rolling gave better survival rate (67 %) compared to those operated 12 hrs, after detorsion awaiting delivery (20 %).

Discussion

Detorsion of uterus was achieved by rolling in 80% buffaloes. The survival rate in buffaloes with completely dilated cervix at detorsion was maximum (95 %), followed by cases with incompletely dilated cervix (67 %) and least in buffaloes with closed cervix at detorsion (50%). This explains that cases occurring late in first stage of delivery when the cervix was fully dilated and in which the stress due to dystocia was relieved by early detorsion, had better chances of survival. Lower survival rate was observed in cases with closed cervix in which the torsion probably occurred just before or at initiation of labour. The survival rate decreased with increase in the time taken for expulsion of fetus following torsion.

In group I, 80% cases were detorted by rolling, all of which survived following successful delivery. These results are in agreement with Mannari and Tadkod (1976) and Pattabiraman *et al* (1979) who reported 100% survival in such cases. In group II, 62% cases survived following caesarean section. Verma *et al* (1974) and Singh *et al* (1978) obtained similar results in directly operated cases. The survival rate was much lower (45%) in group III in which caesarean section followed unsuccessful rolling. Survival rate was lowest (20%) in cases where caesareans were delayed for 12 hours awaiting delivery after detorsion. It appears that stress of rolling together with delay in removal of dead fetus marred post-operative chances of success. Early execution of right type of treatment helped in better survival rate. Fresh and unspoiled cases responded well to treatment in groups I and II.

Table 1 : Details of buffaloes with uterine torsion, their treatment and survival rate. (N = 77*)

Item	Number of buffaloes rolled (Group I) = 55				Number of caesareans done without prior rolling of the dam (group II) = 13	Number of caesareans done after rolling the dam (Group III) = 11**
	Detorsion achieved = 44 (80%)			Detorsion failure = 11 (20%)		
Cervix	Fully dilated 19	Partially dilated 21	Closed 4	Not known		
Delivered	19 (100%)	19 (90%)	1 (25 %)	Nil		
Survival	18 (95%)	14 (67%)	4 (100%)	8 (73%)	8 (62%)	5 (45%)
Taken to group III**	Nil	2	3	6 (Two cases taken away without further treatment)		

* Five buffaloes died before treatment and 4 were taken away by owners without any treatment.

** Buffaloes which failed to deliver following rolling in Group I constituted group III.

Animals of group I which could not be detorted by rolling (group III) were found to have either adhesions or rupture of uterus. The escape of fluids through uterine tear did not allow effective pressure on the uterus for detorsion. It is concluded that rolling is the best treatment in fresh and unspoiled cases of uterine torsion. In cases where two rolls of the animal do not show any progress further rolling should be stopped to avoid further stress to the dam. Caesarean section, owing to its complexities and post operative complications should be reserved only as a last resort.

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Serum Testosterone Levels In Cows With Cystic Ovarian Disease*

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ABSTRACT

Twelve cows of mixed inheritance having cystic ovarian disease (COD) were studied. Rectal palpation and blood collections were made at different intervals to monitor ovarian structures and level of testosterone in blood. Testosterone levels were quite variable (range 10-100 pg/ml) between cows with COD. Ten cows were treated either with gonadotrophin L.H. 3000-6000 i u or dexamethasone 24 mg, i.m. Testosterone levels were observed to be 31.27 ± 4.62 pg/ml at estrus or pre-treatment and 43.04 ± 5.18 pg/ml post-treatment. Statistically, there was no significant difference between pre and post-treatment testosterone levels. Of the 10 cows treated, 6 conceived. Testesterone had no significant role in estrual behaviour in COD cases.

* * *

In normal ovarian follicles, testosterone appears to be produced by theca cells and converted to estradiol by granulosa cells (Fortune

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and Armstrong, 1978). Ovarian cysts are characterized by a thickened theca layer and absence or varying amounts of granulosa cells (Al-Dahash and David, 1977). Synthesis and secretion of testosterone, therefore, may be different in cystic ovarian disease (COD) cases from normal ovarian follicles. There are few reports on testosterone levels in cows with ovarian cysts (Dobson *et al* 1977; Kesler *et al*, 1979). The present study was undertaken to determine the serum testosterone levels in cows with ovarian cysts prior and subsequent to treatment and ascertain its relationship with estrus behaviour.

Materials and Methods

Twelve cows of mixed inheritance (Sahiwal, Red Dane, Sahiwal × Red Dane, Sahiwal × Red Dane × H.F.) of the University farm, with COD were included in this study. All the cows were gynaeco-clinically examined repeatedly to monitor the type of ovarian cyst. Blood serum collections were made pre- treatment while in estrus and at different intervals post-treatment for testosterone asssay and stored at – 20°C until assayed. (Testosterone levels in serum were quantified by radioimuno assay (RIA) procedure as per Falvo and Nalbandov (1974) using highly specific antisera. Intra-assay and inter-assay variation was 9.60% and 11.50% respectively.

Six cows were treated with gonadotrophin L.H. (Biochem Pharma, Bombay) 3,000-6,000 IU as single i.v. injection and four cows were treated using dexamethasone 24 mg as a single i.m. injection. Cows were inseminated in subsequent estrus with frozen semen. Overall conception rate was calculated (1-3 inseminations). Pre-and post-treatment testosterone levels were compared by Student's 't' test.

Results and Discussion

Of the 12 cows, 10 had follicular and 2 had luteal cysts (Table 1). These animals had irregular cycles and were bred many (range 2-12) times prior to this study. Testosterone levels were quite variable and ranged from 10 to 100 pg/ml serum. Cows with follicular cysts had lower levels of testosterone (< 45 pg/ml) compared to luteinized cysts (> 45 pg/ml), except one cow with follicular cyst in which it was 57 ± 16 pg/ml. Similar to this study, Dobson *et al* (1977) reported 78.4 ± 18 pg/ml plasma testosterone in cows with follicular cysts. Also Kesler *et al* (1979) observed plasma testosterone levels ranging from 19 to 146 pg/ml (Mean 60.8 ± 2.7 pg./ ml).

Pre-treatment levels of testosterone in cows with COD were lower (31.27 ± 4.62 pg/ml) compared to post-treatment levels (43.04 ± 5.18 pg/ml.) This increase of 12 pg/ml in treated group was not statistically significant (Table 2). Similar increase in testosterone levels was observed following treatment of COD cases with GnRH, possibly due to luteinization of cysts (Kesler *et al*, 1979). Shemesh *et al* (1975) and Henderson and Swanston (1978) reported that bovine corpora lutes and luteinized granulosa cells are capable of testosterone synthesis in vitro.

Of the 10 cows treated with either gonadotrophin or dexamethasone, 6 (60%) conceived with 1-3 inseminations. These results are lower compared to earlier report (Nakao and Ono, 1977). In conclusion, the levels of testosterone in cows with follicular cysts or at estrus were not statistically different from post-treatment levels (when cows were not in estrus). It appears that testosterone level plays little role in estrus behaviour.

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Table 1 : Variation in testosterone levels in cows with Ovarian Cysts.

Sr. No.	Animal Code	No. of blood samples	No. of heats	Type of Ovarian cyst	Serum testosterone levels pg/ml; Mean ± SE (range)
1.	S 3	7	3	Follicular cyst	39.85 ± 2.64 (25–68)
2.	S 20	4	4	Follicular cyst	22.0 ± 1.73 (20–28)
3.	S 24	3	2	Follicular cyst	57.33 ± 15.75 (19–80)
4.	S 26	5	4	Follicular cyst	21.0 ± 4.99 (10–40)
5.	S 113	2	6	Follicular cyst	30.0 ± 14.18 (10–50)
6.	S 132	3	12	Follicular cyst	41.33 ± 0.54 (40–42)
7.	RD 4	4	8	Follicular cyst	42.5 ± 5.11 (26–52)
8.	RS 101	6	9	Follicular cyst	32.16 ± 10.88 (10–86)
9.	RS 110	5	7	Follicular cyst	29.8 ± 6.31 (13–52)
10.	HRS 58	4	3	Follicular cyst	38.0 ± 14.99 (26–59)
11.	AC 127	5	10	Luteal cyst	75.5 ± 8.43 (52–96)
12.	AC 113	4	6	Luteal cyst	45.5 ± 16.40 (12–100)

Table 2 : Serum testosterone levels in COD cases at estrus or pre-treatment and following treatment with gonadotrophins or dexamethasone.

Status	Serum testosterone levels pg/ml Mean ± SE (range)	Significance (Student's 't' test)
Pre-treatment (estrus)	331.27 ± 4.62 (10–68)	Non-significant
Post-treatment	43.04 ± 5.18 (10–100)	

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CASE REPORTS

IJAR:12:2:203 :1991

Paraphimosis In Nondescript Bullcalf

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Paraphimosis is the condition in which the affected male is unable to normally withdraw the protruded penis into the prepuce due to congenital, psychic or acquired causes. In the present case a non-descript 1 1/2 year old bullcalf was presented to the Veterinary Polyclinic. He attempted to mount a cow in heat with fully erect penis which dashed forcibly against the hind quarters of the cow without reaching the vulva, resulting in trauma with glans penis bent at its base (Fig. 1). There was

Paraphymosis in a Non-Descript bull calf.

haemorrhage and inflammatory swelling of protruded area of glans penis. Initially there was some difficulty in urination but later on it normalised. The bent protruded and inflamed glans penis was cleaned with potassium permanganate (1 : 1000) lotion. Thereafter, ice-cubes were applied with cold fomentation. Thrombophob ointment was applied to the protruded penis. A clean nylon bag was applied over the protruded portion to prevent soiling, injury and maggots. Betnesol 2 ml and Oxysteclin 10 ml were injected I.M. for two days. On the second day it was observed that the oedematous, inflammatory swelling of the glans penis was reduced and it was possible to manipulate the prepucial orifice easily, without any adhesions. The prepucial sheath was irrigated with potassium permanganate lotion (1 : 1000) and prepucial orifice infiltrated with 10 ml of 2% Xylocaine. After lubrication with liquid paraffin, the protruded part was gently pushed into the prepucial sheath. On complete reposition of the protruded glans, the orifice was narrowed by giving two simple interrupted sutures with monofilament nylon. Liquid Terramycin 10 ml was infused into the prepucial sheath and owner advised to keep a close watch. After seven days, the sutures were removed and the response was uneventful.

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Extra Uterine Pregnancy In A Cow

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A non descript grey cow aged 13 years was brought to the Obstetrics unit of the Large Animal Clinic of Madras Veterinary College (LAC. O. 15764) with the history that the animal was pregnant and had tendency for antepartum vaginal prolapse. Rectal examination revealed that the foetus was not in the uterus.

Emergency laparotomy on the left flank was performed. In the abdominal cavity, a live foetus was present with intact foetal membranes. The live foetus was extricated from the membranes and the communication of the membranes with the uterine endometrium through the uterine tear was severed by caesarean section and repaired. The cow died 24 hours following the operation. On P.M. examination, a transverse tear in the left cornua about 6 cms in width, 10 cms away from indistinct body of uterus was observed. Eight maternal caruncles each weighing 400 gms, 7 to 8 cms in diameter were seen on the left cornua and indistinct body of the uterus. Numerous small polypus areas (accessory placentation) were present in the rest of the area.

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Pyometra In A Crossbred Heifer

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A cross bred heifer was brought with a history of insemination four months ago. Mucopurulent vaginal discharge was noticed for last five days. Rectal examination revealed corpus luteum (CL) in the right ovary, flaccid uterus and asymmetry of horns. The condition was differentiated from pregnancy by the absence of double slipping, foetal cotyledons and fremitus. The cervix was relaxed and the pus from vagina was greenish in colour and thick in consistency.

Treatment was given by injecting Prostaglandin F_2 alpha 12.5 mg (Lutalyse, Unichem Labs., Bombay) by intravulvo-submucosal route ipsilaterally and the owner was advised to bring the animal on the third day for further examination. After 72 hrs there was marked reduction in the size of the uterus, CL regressed and nature of discharge improved. According to Katherine Bretzlaff (1987), clinical and microbiological healing is more rapid than cytological and histologic repair and there

may be a 30 to 40 days delay in return to normal fertility even after clinical recovery seems complete. Hence the animal was given a course of Ampicillin (Intrac Phama, Pollachi) along with Tab. Septillin (Himalaya Drug Co., Bombay) @ 10 tablets for 10 days. Since the normal oestrus cycle was restored, sexual rest for two cycles was advised.

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IJAR:12:2:205 :1991

Amputation Of Parasitic Limbs In An Epignathus Calf

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A 3 1/2 months old male buffalo calf having four extra limbs attached to the ventral abdominal wall was presented to the Veterinary College Clinics, Bidar. These accessory limbs were obstructing locomotion of the calf. Clinical examination revealed two fully developed parasitic hind limbs and two underdeveloped parasitic fore limbs with an anus like perforation in between the two parasitic hind limbs. The entire mass was attached to the autosite near the ventral abdominal wall (Fig 1) and the condition was diagnosed as 'Epignathus'.

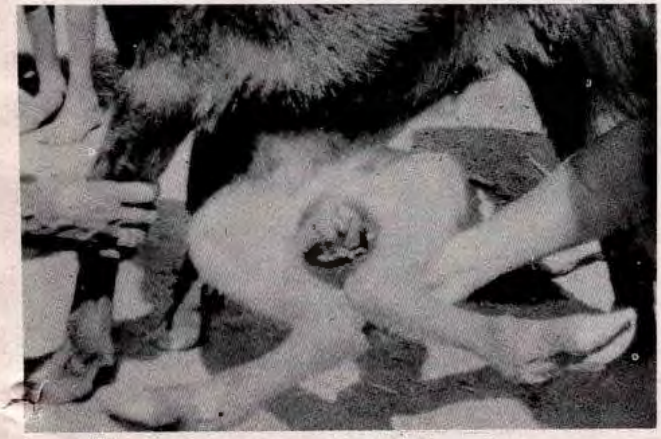

Fig. 1 Epignathus male buffalo calf.

The case was surgically operated to ampute the parasitic limbs but the calf failed to recover following surgery. The clinical examination of the parasitic mass revealed no extensive attachement with the autosite. The anus like perforation encountered between the two parasitic limbs had a small rudimentary intestine like structure distally which had extended into the autosite's viscera without any union with autosite's intestine. However, the parasitic mass had extensive vascularisation.

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Ventral Hysterocoele In A Doe – A Case Report

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There is paucity of literature of the incidence of ventral hysterocoele in goats. The present paper deals with a case of ventral hysterocoele and its successful surgical correction in a doe.

A non-descript full term pregnant pluriparous doe aged about 2 1/2 years was brought to the Madras Veterinary College hospital, with intermittent straining and signs of parturition since 6 hours. The animal was reported to have met with an accident during second month of pregnancy. Vaginal examination revealed two finger dilation of the cervix. There was a big swelling on the right side of the abdomen. The swelling was soft and extended downwards deviating the udder (Fig. 1). Palpation of the mass revealed fluid thrill and foetal parts. The mass was irreducible. Radiological examination of the right flank revealed the presence of foetal skeletons inside the hernial sac. Hence, the case was diagnosed as ventral hysterocoele and prepared for cesarean section and herniorrhaphy since normal vaginal delivery was not possible.

Adopting aseptic precautions, the right flank area over the hernial sac was prepared for surgery under local infiltration with 2% lignocaine, Hcl. An oblique cutaneous incision of about 15 cms was made on the hernial sac. The right gravid horn of the uterus was seen protruding through the hernial ring of 9.5 cm diameter. A longitudinal incision was made on

Fig. 1 Ventral hysterocoele in a Doe.

Fig. 2 Surgery on the right flank area over the hernial sac.

the uterine horn and two normal, live male kids were relieved alongwith foetal membranes. Uterine incision was closed by inversion suture using chromic catgut. The uterus was then replaced into the abdominal cavity through the hernial ring. The edges of the hernial ring

Fig. 3 Uneventful recovery of the doe

(Fig. 2) were freshened with scalpel blade and opposed with No. 2 chromic catgut by interrupted overlapping (sliding) mattress pattern suture. The suture line was reinforced with a row of simple continuous pattern with the same material. The skin was sutured with black silk in routine manner. The animal was administered parenteral fluids, antibiotics, cortisones and intrauterine antibiotic infusions for next 5 days The cutaneous sutures were removed on day 10, post operation and the healing was found satisfactory with no abdominal swelling and the animal had made an uneventful recovery (Fig. 3).

Extensive unilateral ventral hysterocoele in ruminants is one of the maternal cause for dystocia mainly due to the inability of the abdominal muscles to contract equally. In the present case, besides this factor, the two kids were inside the portion of herniated uterine horn. The size of the uterus along with its contents was almost twice the size of the hernial ring and hence the fully grown foetus could not have passed through the hernial ring. Thus, in this particular case, cesarean section was fully justified to relieve the live foetus and also save the life of the doe. Benesch and Wright (1957) also advised cesarean section in case of ventral hysterocoele. Overlapping (sliding) mattress sutures adopted in this case was found to be ideal for ventral herniorrhaphy (Oeheme and Prier, 1974.)

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Schistosomus Reflexus In A Sheep-A Case Report

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A Madras red cross sheep aged 3 1/2 years was presented with the history of full term pregnancy and straining since last 6 hours. Vaginal examination revealed complete cervical relaxation and all four foetal limbs presented in the birth passage, with exposure of visceral organs. After thorough lubrication, a dead lamb with foetal membranes was extracted per vaginum. Routine antibiotics cover and supportive therapy were administered for 5 days and the animal had an uneventful recovery.

The dead female monster weighed 2 Kgs. The spinal column was reflected dorsally and cranially tending to bring the sacrum in approximation to the cranium. The spine was ankylosed and rigid. The abdominal wall was bent dorsolaterally, the cavity was open and all the visceral organs exposed (Fig. 1) The foetal liver was abnormal in shape, whereas other viscera appeared macroscopically normal. All limbs were deformed and long axis directed cranially.

The monster was a typical Schistosomus reflexus as per the classification of Arthur *et al*, (1989). It is considered to be a severe form of abdominal hernia associated with skeletal defects, which results in monstrosity.

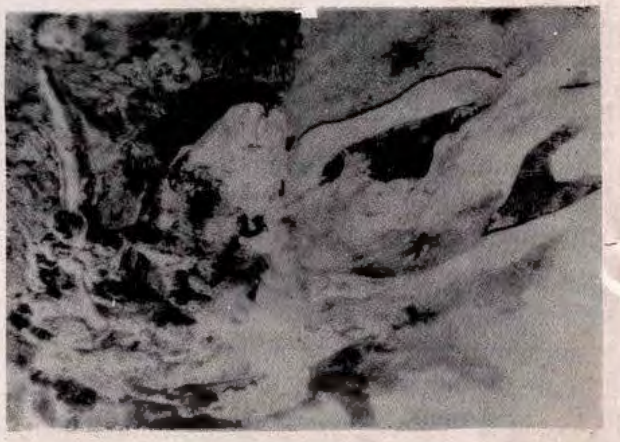

Fig. 1 Schistosomus Reflexus in a sheep.

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Ovarian Dysgerminoma In A Pomeranian Bitch

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Dysgerminoma is a malignant gonadal tumour originating in the dormant undifferentiated cells of the ovary or testis. A rare case of canine ovarian dysgerminoma is reported.

Case History

A Pomeranian bitch aged 14 years was presented in the College Hospital with the history of irregular oestrus cycles and sterility.

Clinical Findings – The bitch was normal, healthy and of aggressive temperament. Abdomen was moderately pendulous with its ventral surface hyperpigmented. Deep palpation revealed firm and distended uterine cornua with distinctly hard tumour like mucoid or mucopurulent vaginal discharge. A tentative diagnosis of ovarian tumour was given and surgical removal advised.

Treatment – Panhysterectomy operation was performed under general anaesthesia. The post-operative recovery of the bitch was uneventful. Examination of the genitalia revealed firm, distended and enlarged uterine cornua with an encapsulated tumour in the left ovary. Right ovary was normal. Metastasis was absent.

Results and Discussion

The tumour measured 26 cms at its greatest diameter and weighed 400 gms (Fig. 1). The tissue on cut surface was homogenous and soft. Histopathological findings revealed the cells of variable size with dense nnclear

Fig. 1 Genitalia of a bitch showing tumour on the right ovary. Note distension of the uterina cornua.

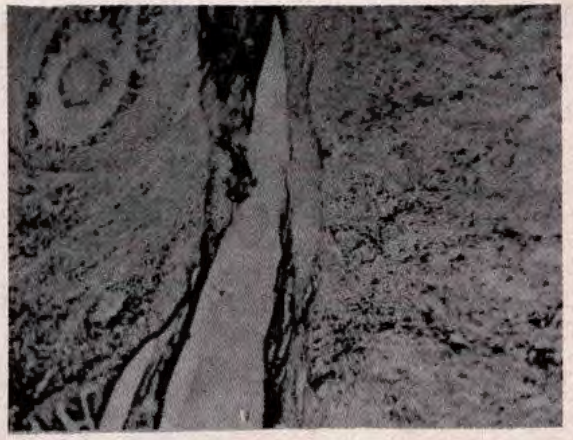

Fig. 2 Photomicrograph of ovarian dysgerminoma showing atretic follice. Invasion of ovarian tissue by fibrous connective tissue is also seen (H and E 100x)

chromatin indicative of differentiation towards spermatocytes and lamellar pattern reticular fibres suggestive of tubular formation, confirming it as dysgerminoma (Fig. 2)

Dysgerminoma is usually unilateral (90%), with a greater left sided frequency (50%), than the right (35%). In the present case also it was left sided, allied to the observations of Robbins (1974). Dysgerminoma is not known to secretc hormones (Smith and Jones 1972). Extensive displacement of normal functional ovarian tissue appears to hamper secretion of oestrogens, lack of which results in irregular oestrus. The hyperpigmentation noticed in this case may be due to increase in melanophore stimulating hormone due to pituitary adrenal upset, secondary to the ovarian tumour.

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Sertoli Cell Tumour Of The Testis Of A Bullock

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A bullock aged about 8 years and castrated earlier at a young age, was presented with the complaint of large pendulous swelling at the scrotal region, because of which it was difficult for the animal to walk properly.

The animal was restrained and on palpation the mass was found at the site of right testis. It was irregular in shape and had fibrotic consistancy lacking any tone. The left testis was small castrated mass like.

To cure the malady, it was decided to remove the mass. The animal was tranquilised with 60 mg Triflupromazine (Sequil, Sarabhai Chemicals, Vadodara) followed by anterior epidural anaesthesia with 30 ml of 2% Lignocaine Hydrochloride (Xylocaine, Astra-IDL Ltd; Bombay) solution. The operation to remove the mass and left testis was performed on lateral recumbancy giving 2 separate incisions as described by Pandey *et al* (1989). The scrotal incisions were kept open. The cavities were dressed with Oxytetracycline liquid (Terramycin, Pfizer India Ltd; Bombay). Streptopenicillin (Bistrepen, Alembic, Vadodara), 2.5 g, was injected I/m, one day pre and 5 days post- operation. Prednisolone (Hostacortin-H, Hoechst, Bombay), 100 mg/day was injected I/m for 3 days post-operation. The wound healed within 25 days without any complications. Subsequent follow-up revealed that the animal regained its normal walking gait and performed routine draught work.

On clinico-pathological examination the mass revealed as sertoli cell tumour of the testis. The shape of the right testis was irregularly oval of 490 g. weight, 20.50 cm length and 10.50 cm. width. It was lobulated and enclosed in a tense tunica albuginea. On cutting, its surface buldged. The mass was fibrotic and firm (Fig. 1).

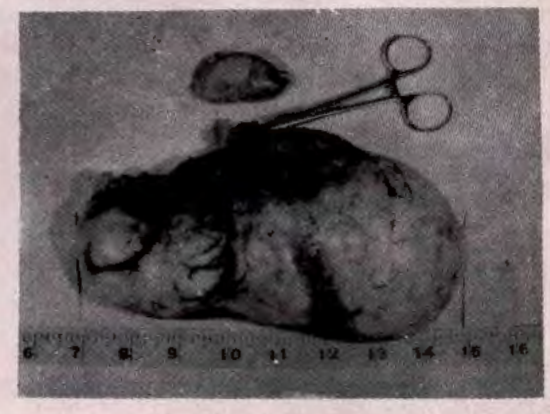

Fig. 1 The enlarged right (below) and castrated left testes of a bullock.

Jubb *et al* (1985) stated that in bulls testicular tumours are seldom observed except in old animals. This is more so because in larger animals relatively few uncastrated males are kept until they reach an advanced age. They attributed hardness and enlargement of the testis with sertoli cell tumour to the abundance of stroma. The present case appears to be due to incomplete castration. McEntee (1970) also reported a case of sertoli cell tumour in the testis of a bull. Roberts (1971) stated that the tumour arises from the sertoli cell of the seminiferous tubules and if no metastasis have

occurred, the recovery is very prompt on removal of the affected testis, as in the present case.

Acknowledgement

Thanks are due to the Head, Pathology and the Dean of the College for the facilities.

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Clinico-Pathological Studies On Canine Testicular Neoplasms

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Testis retained within the body have a strong tendency to neoplasms. Such dogs are often of uncertain disposition (Whitney, 1948). Dogs with one testis in the scrotum and one undescended are fully fertile, but seldom sire cryptorchid pups (Marca Burns, 1952). Probably the highest incidence of testicular neoplasia is in the dog. Three types are common : seminoma, interstitial cell tumour (ICT) and sertoli cell tumour (SCT). More than one histological type, may be present in a single testis (Jones and Joshua, 1982).

Clinical changes associated with testicular neoplasms have not been fully described. Gynecomastia, alopecia and pendulous penile sheath have been associated with sertoli cell tumour (Lipowitz *et al*, 1973). However, the information of tumour types is lacking in available literature. Present study pertains to the major disorders clinically associated with testicular neoplasms in dogs.

Materials and Methods

The material comprised of 53 male dogs of assorted genetic makeup brought to the College Hospital for routine castration, orchidectomy due to cryptorchidism or clinical disease due to hormonal imbalances.

Results and Discussion

11 (18.97%) dogs had testicular tumours (Table 1). It is obvious that interstitial cell tumour (8/11 cases) is the most common (72.73%) testicular tumour in mongrel dogs, occurring at an average age of 8 years. This

supports the earlier findings of Prange *et al* (1986) that interstitial cell tumour is most common in dogs aged 8.3 years. The prevalence of interstitial cell tumours was found in the mongrels since they outnumbered other canine breeds studied in the present investigation. Clinical manifestations of ICT varied. Distinct findings were : hyperkeratosis, non-responsive pruritus, easy epilation of hairs, seborrhoea, and unequal sized testis. It is evident from the clinical picture that overt manifestations of this tumour could not be generalized probably because of hormonal imbalances. Lipowitz *et al* (1973) also reported that hormonal imbalances do not occur in dogs with ICT. Moulton (1961) was of the view that though interstitial cells of the normal animal secrete male hormone and are responsible for the secondary sex characteristics, tumour cells have no hormonal effects, libido remained unaffected without priapism. Histo-morphological examination revealed large cells with numerous fat droplets. Mitotic figures were not conspicuous. Runnels *et al* (1965) opined that ICT seldom became malignant and metastasized and hence the prognosis is favourable. However, there was no clinical evidence or manifestation of metastasis encountered in the present studies.

Sertoli cell tumour (SCT) was recorded in only two (18.18%) cases. The distinct clinical changes associated with this tumour type were alopecia particularly on the ventral aspect of body, posterior aspect of thigh, hairs could be epilated easily, hyperpigmentation of skin in the inguinal region, absence of pruritus, scaling and excessive enlargement of the nipple. Similar observations have also been recorded by Damodaran *et al* (1974). More overt clinical changes are associated with sertoli cell tumour (Lipowitz *et al* 1973). Altered hormone production by the neoplastic testicle and possibly the pituitary gland and a variation in response by target organs may be responsible in part for the clinical changes associated with testicular tumours. In SCT, feminization occurs with greater frequency when the tumour is in the extra-scrotal position. In the present study also, in one case aged 7.4 yrs, the left testicle retained in the abdomen developed tumour. However, similar findings could not be confirmed in the cryptorchid at the age of three years with clear indications that tumorous development occurs in advanced age. Alopecia which is the most early recognizable manifestation and about which the dog owners are inquisitive, is mainly due to atrophy of hair follicles, whereas, dry coat and possibly the pruritus are due to atrophy of sebaceous glands (Moulton, 1961). The incidence of tumour is significantly higher in retained testis than in normally descended gonads, for which reason removal of undescended testis is advisable before neoplastic change is likely to occur (Relf and Brodey, 1969). In the present investigation removal of undescended as well as descended testicle caused remission of the symptoms and cutaneous improvement. Sertoli cell tumour by far the most clinically significant of the canine testicular tumours, is frequently endocrinologically active producing a wide range of sex hormones from androgens to oestrogens and occasionally metastasizes (Lindberg *et al* 1976). Microscopically the SCT displayed rarity of spermatogenic and interstitial cells. Neoplastic cells formed solid infiltrating cords.

Seminoma which is the most malignant of the testicular tumours is fairly common in dogs (Moulton, 1961). However, a solitary case (9.00%) of adeno-carcinoma (seminoma) is recorded in this study. Alterations in secondary

sex characteristics as evidenced in SCT were absent in canine seminoma. This clearly suggests that oestrogen is not produced by this tumour. Histo-pathological examination of the affected testis revealed spermatogonial cells with nuclei (Fig. 1). The tumour cells are fairly uniform in size and shape. Bomhard *et al* (1978) studied ultrastructure of testicular tumours and reported that intra-cellular bridges were absent in seminoma.

It may be concluded from this study that overt clinical manifestations of symmetrical alopecia, gynecomastia and cutaneous hyperpigmentation are liable to remission on removal of the testes.

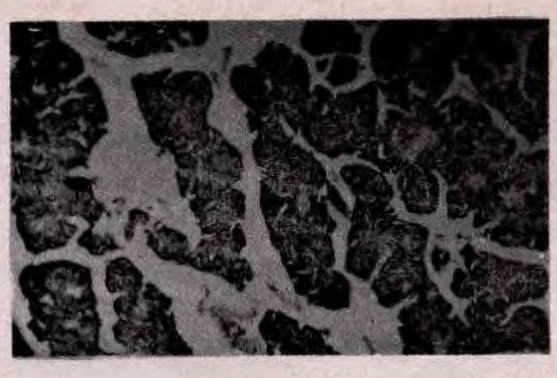

Fig. 1 Photomicrograph of testis showing spermatogonial cells and prominant nucle (H and E 100 x)

Table 1 : Frequency of testicular tumours.

Breed	No. of dogs	Mean age in years	Number of dogs with tumours		
			Interstitial cell	Sertoli cell	Seminoma
Mixed / Mongrel	8	8.3	8 (72.73)	–	–
Alsatian	2	5.6	–	1 (9.09)	1 (9.09)
Pomerarian	1	7.4	–	1 (9.09)	–
Total	11	7.0	8 (72.73)	2 (18.18)	1 (9.09)

Figures in parenthesis indicate percentage.

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WILD LIFE

IJAR:12:2:215-216 :1991

Studies On Certain Aspects Of Reproduction In Lioness (Leo Felies) In Captivity

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ABSTRACT

Sexually mature seven lioness and three lions in captivity were studied for some aspects of reproduction. Lioness were monoestrus animals. 57.14% lioness exhibited oestrus once a year and 42.86% twice a year. 45.45% lioness exhibited heat in monsoon, 31.81% in winter and 22.71% in summer. Average duration of heat was 7.6 days. Average gestation period was 102.5 days and cubs per litter was 4. The cubs were born spotted and striped, with closed eyes. Sexual behaviour, courtship and copulation were studied. One case of pseudopregnancy was recorded.

* * *

Wild life helps in the maintenance of ecological balance of nature. However scant attention is paid to reproduction in wild life. Present studies on lioness (Leo Felies) in captivity were therefore undertaken.

Materials and Methods

Seven lioness and three lions belonging to Maharaj Bag Zoo, Nagpur were observed over a three year (1980-83) period to study certain aspects of reproduction : (i) Oestrus and Oestrous behaviour, (ii) Sexual behaviour, (iii) Courtship / coitus (iv) Gestation period (v) Parturition, (vi) Number of cubs born per litter, and (vii) Pseudo-pregnancy.

The lioness and lions were sexually mature and identified by numbers painted in thigh region. Lioness were kept in a group of three with one lion in each cage.

Results and Discussion

(i) *Oestrus and Oestrus behaviour* : Lioness 1, 3, 5 and 7 exhibited oestrus once a year where as lioness 2, 4, 6 exhibited oestrus twice a year. Thus four lioness (57.14%) showed two seasons in a year and 3 lioness (42.86%) showed only one season in a year. These observations are in full agreement with Hanmvas (1963) but differ from that of Asdell (1946) who reported that lioness were polyoestrus animals.

During the period of three years of study, 45.45% oestruses were observed in Monsoon (June to September) 31.81% in Winter (October to January) and 22.71% in Summer (March to May). The average duration of oestrus was 7.6 days (range 4 to 9 days). This is in full agreement with Hanmvas (1963) who stated that breeding season is not restricted to any particular period of the year.

(ii) *Sexual behaviour* : Lioness in oestrus remained off feed for 2-3 days and got segregated in a corner and remained with a male during oestrus period. The vulval lips were turgid, swollen and moist with frequent micturition. The lioness in heat tried to attack

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the zoo keepers. This is in agreement with Heint Hedger (1963) who observed similar sexual behaviour during breeding season.

(iii) *Courtship and coitus* : Lioness in heat licked the ears, face and loin region of the male that accompanied her and made typical mating call during the courtship. The pair walked a short distance and then the female sat on the floor. Lion kept the forelimbs on the sides of the abominal region of the lioness for mating in the squatting position.

The coitus in this species was very typical. Lioness sat on the floor and moved her tail on one side, slightly raising her hind portion. The lion thereafter mounted the lioness in heat and kept his forelims on the ground, just on the two sides of her abdominal region. The friction type coitus lasted one to two minutes. The frequency of coitus ranged from 80-120 times in a day.

(iv) *Gestation period* : Average gestation period was 102.5 days (range 99 to 104 days). The gestation period in the domesticated lioness is more than 62 days as per Asdell (1946), but nearer to 106 days and 92-113 days as recorded by Walker *et al* (1964) and 105 days (Hanmvas, 1963). The difference in gestation period may be due to variation in counting the length of oestrus and the exact time of conception. Similar problem was encountered by Asdell (1946).

(v) *Parturition* : Pregnant lioness due for parturition was isolated to a separate, undisturbed cage. A grass bedding was provided and the room kept dark and in such condition only the lioness gave birth to cubs. There were difficulties in studying the process of parturition. Present observations are in full agreement with that of Jewell and Loizos (1964).

(vi) *Litter size* : During the period of study, lioness 1, 2, 3 and 7 delivered 15 (8 male and 7 female) cubs with an average of 3.7 (range 1 to 3) cubs per litter. The cubs born were spotted and striped with closed eyes. This is in full agreement with Steyn (1967), Ledekher (1963), Hanmvas (1963) and Walker *et al* (1964).

(vii) *Pseudo-pregnancy* : One lioness (No. 5) manifested symptoms of pseudo-pregnancy. During this condition, she acquired more fat and lustre on the body and showed engorgement of teats. Normally such changes were observed prior to parturition only. This condition was observed 75-80 days post-oestrus even if the lioness was not served. After this period was over, the teats were reduced in size and she lost lustre and weight. The lioness did not deliver cubs and hence a case of pseudo-pregnancy. Some lioness exhibited pseudo-pregnancy in subsequent breeding seasons also.

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ISSAR News

The Tenth National Convention of ISSAR will be held at the Madras Veterinary College, Madras from March 19-21, 1992. Please refer the First Announcement published elsewhere in this issue for all details.

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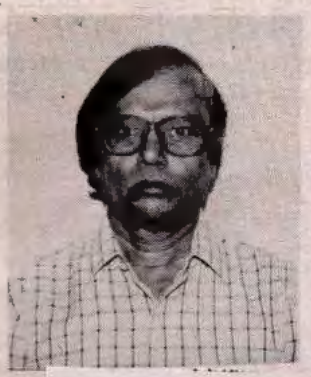

Dr. P. B. Kundu

Dr. P. B. Kundu, Director of Veterinary Services, West Bengal is appointed Animal Husbandry Commissioner, Govt. of India w.e.f. 24-6-91.

His long and rich experience in the field of Education, Research and Administration will be an asset for the speedy advancement and all round progress of Animal Husbandry Sector at the National level. We congratulate Dr. Kundu, wish him every success and assure him of our best co-operation.

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It is with immense pleasure we learn that Dr.Allaudin Ahmed, Vice-Chancellor, S.K. University of Agriculture and Technology, Kashmir and an eminent Veterinary Educationist of repute is appointed Deputy Director General(Education), ICAR, New Delhi. We wish him best of luck.

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Dr. B. N. De

Dr. B. N. De, Joint Director is appointed Director of Veterinary Services, West Bengal, Calcutta w.e.f. 21-6-91.

He is a dynamic talented researcher and administrator of repute. Incidentally he is Joint Secretary, ISSAR taking keen interest in its activities. We congratulate Dr. De and wish him all success

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We welcome the following appointments, postings:

Dr. J. Rajasekaran, Professor and Head, Department of Obstetrics & Gynaecology, Madras Veterinary College, Madras. He is the Organising Secretary of the 10th National Convention (ISSAR), hosted by Tamil Nadu University of Veterinary and Animal Sciences at Madras, March 19-21, 1992. We extend all co-operation to make it a triumphant success.

Dr. F. S. Kavani, Professor and Head, Department of Gyanecology & Obstetrics, Veterinary College, GAU Campus, Sardar Krnshinagar, w.e.f. 6-5-91.

Dr. Y. G. Dugwekar, Professor and Head , Department of Gynaecology & Obstetrics, Gujarat Veterinary College, Anand.

Dr. F. S. Chauhan, Research Scientist(Embryo Transfer) Reproductive Biology Research Unit, GAU, Anand w.e.f. August 1991.

Dr. V. L. Deopurkar, Professor of Gynaecology, Bombay Veterinary Collegge, Parel, Bombay-12.

Dr. R. K. Pandit, Associate Professor, Mhow Veterinary College as Head, Deptt. of Obstetrics & Gynaecology, College of Veterinary Science & A. H., Jabalpur(M.P.)w.e.f. 24-10-91.

Dr. A. S. Nanda, Professor cum Head, Department of Obstetrics & Gynaecology, College of Veterinary Science, PAU, Ludhiana w.e.f.23-11-91.

We extend our heartiest congratulations to them all and wish them a bright future.

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Dr. K. G. Kharche, Professor & Head, Deptt. of Obstetrics & Gynaecology, Jabalpur Veterinary College retired on 30-6-91 after rendering a very active and meritorious service. His keen interest in ISSAR activities in general and IJAR in particular is deeply acknowledged. He has settled at Malkapur(Buldana) in Vidarbha.

We wish Dr. Kharche and his family a very happy, prosperous and active retired life. His rich experience will be an asset to ISSAR in future.

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ISSAR Gujarat Chapter organised :

A clinical camp for Reproductive problems of cross-bred cows of Devol Milk Producers Coop. Society oh 7-4-91. Free Veterinary aid was rendered to 95 problem cases by Drs. S.B.Kodagali, H. J. Derashri, J. S. Patil and J. H. Prabhakar.

A one-day workshop on 'Current problems of Reproduction' at Galbabhai Dairy Coop. Training Centre, Palanpur on 4-8-91 for over 30 Veterinary Officers of Banaskantha District Coop.Milk Producers Union Ltd. Drs. S. B. Kodagali, Y.G. Dugwekar, H. J. Derashri, F. S. Kawani and G.M.Siddiqui formed the Faculty of the day.

Obituary

Dr. S. K. Sinha

As we go to the press, we learn about the sudden,untimely and sad demise of Dr. S.K.Sinha, Ph.D. FRVCS(Sweden), Professor cum Head, Deptt. of Gynaecology & Obstetrics, RAU, Bihar Veterinary College, Patna on 13-8-91. He was Life Member of ISSAR.

Dr. Sinha with his pleasing manners and scholastic interest was widely known. It is an irrepairable loss to our profession. We extend heartfelt condolences to the bereaved family.

May his soul rest in eternal peace.

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FROM SECRETARY'S DESK

Dear Members ,

As you are aware that our Society has instituted various Awards. I arranged for an announcement regarding the same through various Journals / Publications. I also tried to approach you through your respective State Chapter Secretaries and also through the Heads of the Department of Animal Reproduction of all the Veterinary Colleges in the country. I am delighted to receive very encouraging response in the matter.

Award Committees were already constituted and I have despatched all the articles to the respective Chairman/Members of the Award Committee. I am sure that the Chairman/Members will be critically evaluating the entries and their final verdict will be available in another month's time. As usual, names of the Award Winners will be announced at the inaugural function of ensuing National Symposium of our Society (on 19th March 1992 at Madras). However, I shall be happy to communicate the Award winners individually well in advance, so that, they can receive the coveted Awards at Madras.

Once again I have submitted 30 copies each of application form to the Indian Council of Agricultural Research New Delhi, with a request to provide financial assistance to our Society for publication of the Indian Journal of Animal Reproduction and also for holding National Symposium. Hope that the applications are under active consideration of I.C.A.R. and the Society will receive some financial assistance very soon.

Hope to meet many of you at Madras Conference in March 1992. Till then, I send Season's Greetings and wish you Happy and Prosperous New Year.

11th Nov. 1991
Parbhani.

D.R. PARGAONKAR
Secretary, ISSAR

BOOK REVIEW

"Reproductive Disorders in Indian LiveStock" By : Dr. A. Ramamohana Rao, Dean, Post Graduate Studies, APAU, Hyderabad - 500 030. 1st Edn. March 1991. Pub. Publications and Information Division, Indian Council of Agricultural Research, Krishi Anusandhan Bhavan, New Delhi - 110 012. Chapters 16, Pp. 86 Price Rs. 20.

This is a concise digest comprising of reproductive disorders in female (Part I) and male (Part II) Indian livestock. Latest references are given at the end of each Part. Chapters deal with affections of the gonads and genitalia, repeat breeding, gestational and parturient disorders, infectious infertility, libido, impotentia coeundi and impotentia generandi. Besides, there are Chapters on reproductive disorders in Sheep, Goats and Pigs.

The author widely known for his expertise in animal reproduction has done a yeomen service by abridging latest uptodate research information of vital importance to all field veterinarians in general and research students in particular.

This low price book is a must for every worker in animal reproduction, besides College, University and Institution / Department Libraries.

– A.S. KAIKINI

INDIAN JOURNAL OF ANIMAL REPRODUCTION

ATTENTION

Life Members (ISSAR) and Subscribers are requested to inform change(s) in their address to ensure regular supply of IJAR.

Chapter Secretaries (ISSAR) are requested to inform the names and addresses of Annual Members with period of their Membership for sending IJAR to them.

Nagpur,
December 15, 1991

Dr. A. S. Kaikini
Editor, IJAR