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THE INDIAN JOURNAL OF ANIMAL REPRODUCTION

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CONTENTS

Editorial

Sire Selection In Dairy Cattle Breeding—Possible Impact Of Newer Reproductive & Genetic Engineering Techniques*

Majority of the countries in the region can be proud of the number and diversity of their cattle, for example, the current population of cattle in India is 191 million of which approximately 40% are working animals and only 32% are breedable females. The cattle contribute approximately 40% to the total current annual milk production of 38.8 million tonnes. The majority of cattle in India are non-descript, although we claim to have 26 descript breeds--milch, draft and dual purpose. There is very little field recording of performance data and their utilisation in progeny performance is confined to organised farms which also have rather small breedable female population limiting the number of sires to be tested and the numbers selected from them. This reduces both the accuracy and intensity of selection. There is further serious confounding of the herd environment and the sires, as sires are essentially tested in individual herds. As the number of sires tested is few, there is tendency to utilise majority of them, thus allowing little selection. Since milk production is not the only character usually considered in selection, it further reduces the intensity of selection for milk. Considering that majority of females are needed for replacement specially due to lower rate of reproduction in Indian breeds, there is hardly any chance of bringing genetic improvement through selection of females. The sire selection based on their progeny performance is, therefore, the only system of making genetic improvement in dairy animals.

Instead of my going into the different methods of progeny testing and complicated mathematics associated with it, I will like to confine myself to the possible impact of reproductive and genetic technologies specially related to artificial insemination, embryo transfer technology and genetic engineering. The breakthroughs in reproductive and genetic teehnologies over the past few decades has made it possible to bring much faster genetic progress than was possible through selection and natural service. This has more recently been reviewed by Fitzhugh (1983).[1]

The major directed change in gene frequencies arises from selection and to a limited extent through immigration (introduction of exotic gene through crossbreeding or replacement of local population with the exotics). Even in these two cases i.e. evolutionary crossbreeding & complete replacement, further improvement will have to be brought through selection. The selection is generally defined as a differential rate of reproduction among individuals. Until recently the regulation of reproduction processes of livestock was largely limited to determining which sire should be mated to which dam. The major improvement in the rate of genetic progress has arisen through the possible use of artificial insemination. The use of AI specially with frozen semen has allowed the production of daughters in many herds/locations and comparison of their daughters' performance against contemporaries in different locations and removing the confounding of sire and herd effects as in case of natural service.

1 Fitzhugh, H.A. 1983—Genetic aspects of germplasm storage & genetic engineering—FAO Animal Production & Health paper 44/2—FAO—1984.

The differential impact of the various types of genetic engineering on rate of genetic change such as through AI, sex control, super ovulation & embryo transfer has been studied more recently. The expected rate of genetic progress (ΔG) is a function of the correlation between actual and predicted additive genetic value ($r_{gg'}$) and standardised selection differential (G) and additive genetic standardisation deviation (σg). This progress (ΔG) divided by the generation interval (gL) will provide rate of genetic improvement per year. The genetic progress arises from selection of sires and dams and passes to the next generation from sire to the sons, sire to daughters, dam to the sons and dam to daughters (Table 1).

TABLE 1 Pathways for genetic progress through selection

Selected Parents	Genetic Difference	Generation Interval
Sires of sons	Δ SS	L_{ss}
Sires of daughters	Δ SD	L_{sd}
Dams of sons	Δ DS	L_{ds}
Dams of daughters	Δ DD	L_{dd}

The within herd selection of dairy cattle for milk yield with natural service could best give an annual genetic progress of 0.5 to 0.6 per cent of the mean. Through the utilisation of artificial insemination it is possible to increase it to 2-3 per cent. Although what normally has been achieved is around 1.5 per cent. The major improvement in the rate of genetic progress through artificial insemination has been through increasing the intensity of selection specially in the sires and thus greater contribution through the path-ways from sire to the sons and sire to the daughters. The reduction in the number of dams required for producing prospective sires has also increased the genetic progress through this pathway. The accuracy of the selection as indicated ealier is also improved by comparison of the daughters of the sires in larger number of environments. The non-attainment of the rate of genetic progress of 2-3 per cent per annum is possibly due to other traits not related to milk production included in the selection and non-random management of cows.

The technologies of super ovulation & embryo transfer can provide additional genetic gains than possible through artificial insemination. Two strategies, the juvenile scheme and adult scheme, have been studied. The first involves selection among transferred sons and daughters based on available records on their dams or other relatives and the other involving delayed selection until transferred sibs are three years old and atleast three records of the dam and first lactation record on the transferred female is available. Both the strategies decrease the generation interval from 6.3 years in conventional progeny testing scheme to 1.8 and 3.7 in the two schemes. The maximum increase of 189% arises from juvenile scheme with 32 donor dams per sire and 16 transferred progenies per donor. However, for a fixed number of transfers say 1000 there is a sharp increase in rate of in-breeding. The combination of AI with multiple ovulation embryo transfer will further increase selection differential through dam pathways allowing the best cows to produce sons and daughters for the next generation. Sexing of semen & embryos and embryo cloning would increase genetic progress further (Table-2).

TABLE 2: Predicted Effects of AI, Sexed Semen and Embryo Transfer on Selection Intensity of Sires and Dams and Annual Genetic Gains in Milk Yield: (1)

Intervention	Percent selected				Gain/year
	SS	SD	DS	DD	Kg
Artificial insemination	4	20	6	90	100
Sexed semen	4	20	3	45	115
AI with embryo transfer	4	20	1	10	135
Embryo transfer—intense sire selection	2	2	1	10	159
Embryo transfer, intense sire selection, sexed semen	2	2	0·5	5	167

(1) Genetic standard deviation/Sum of generation intervals = 568 kg./24

In developing countries in which the organisation of a national progeny testing programme is either non-existant or is meagre in size, the scheme involving super-ovulation & embryo transfer, might have special practical relevance as a means of bringing genetic improvement. This techniqne can also help in the conservation of indigenous genetic resources. This aspect is becoming important with the realisation that the indigenous breeds though not as productive as the introduced breeds or their crosses with indigenous breed but are superior in draft and are better adapted to more harsher conditions, possibly possess some unique genes connected with adaptation to harsher physical environment, poor quality feed and resistance to tropical diseases, are required must be conserved and possibly utilised.

The reproductive and genetic technologies under wider name genetic engineering provide great opportunities to improve dairy cattle production through increased selection intensity, shortening of generation intervals and producing new genetic combinations to take advantage of the complementarity and possibly heterosis. However, much work remains to be done before the full benefit of these biotechnologies can be realised. Most important is the need to characterise production systems, resources and contraints to determine which genotypes are best suited to each system.

Editorial Board

*Source: Special lecture delivered by Dr. R.M. Acharya, Deputy Director General (AS), Indian Council of Agricultural Research, New Delhi, at the First Asian Congress on Animal Reproduction held at Bombay from 11th to 13th December, 1985.

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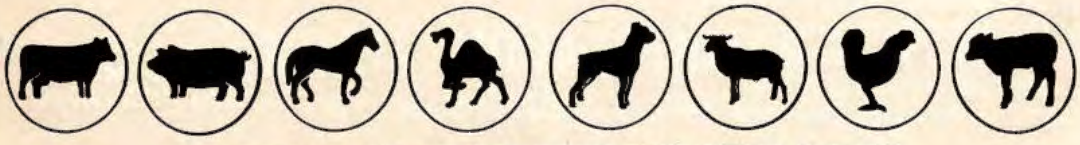

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Spermatozoan Losses During Freezing In Mehsana & Murrah Buffalo Bulls.

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ABSTRACT

The study involved deep freezing of 423 semen ejaculates from seven Mehsana buffalo bulls & 387 ejaculates from eight Murrah buffalo bulls. Fifty ejaculates (10.57%) of Mehsana & 48 ejaculates (10.79%) of Murrah buffalo bulls were found to be either non-motile or had a post-thaw motility below 35% and had to be discarded resulting in to loss of valuable germ plasm. There was a great variation (from zero to 33.33% of ejaculates) among buffalo bulls when preponderance of such ejaculates was cons idered indicating that the semen from all the buffalo bulls was not equally freezable. Sperm losses were high (23.04% for Mehsana & 23.48% for Murrah buffalo semen) when the semen was frozen during Hot season (March-June) compared to other seasons.

* * *

Introduction of frozen semen technology in buffalo breeding programme has accelerated the pace of genetic improvement in these animals. Frozen semen has many advantages over Chilled semen. However, semen from all the valuable bulls can not withstand the rigours of freezing and thawing procedures equally. Incidence of semen ejaculates which are initially non-motile and which do not recover on further dilution and of ejaculates which have satisfactory initial motility but poor post-thaw recovery is quite variable. A significant percentage of motile spermatozoa are lost during freeze-thaw procedures. In the present study an attempt has been made to know the incidence & occurrence of initially non-motile or poorly freezable semen ejaculate & to ascertain spermatozoan losses during freezing procedure in Mehsana & Murrah buffalo bulls.

Materials and Methods

One year study involved 423 normally freezable & 50 flat or poorly freezable ejaculates from 7 Mehsana buffalo bulls (4-5 yr. old) and 387 normally freezable & 48 flat or poorly freezable semen ejaculates from 8 Murrah buffalo bulls (10-12 yr. old) located at Central Semen Collection Station, Mehsana. Semen was collected using artificial vagina. Buffalo bulls were on a collection schedule of 2-3 ejaculates per week and were under identical care, nutrition and management conditions, which included two cold shower baths during Hot summer months. After evaluation for macroscopic and microscopic characteristics the semen ejaculates having initial motility over 70% were diluted in Tris-fructose egg yolk-glycerol dilutor

* Officer-in-charge, Central Semen Collection Station, Mehsana.

and frozen as per Vasanth (1979). An equilibration time of 4-5 hours at 5°C was provided before freezing. Post-thaw motility was recorded during 24 hours post freezing. Loss in initial motility after freezing was recorded. Effect due to bulls, breeds, months & seasons were studied. Conception rates were obtained from the inseminated and followed up cases for each of the buffalo bulls. Statistical analysis of the data was carried out as per Snedecor & Cochran (1967) on Spactrum-7 Computer belonging to the Computer Cell of the Dept. of Agricultural Statistics, B.A. College of Agriculture Anand. The climatic data has been given in Table 1.

TABLE 1: Climatic factors during different months (Means).

Month	Maximum temperature °C	Minimum temperature °C	Relative humidity %
March	37.12	23.00	35.00
April	40.60	26.33	39.00
May	40.83	28.00	55.00
June	40.17	29.33	75.00
July	32.84	23.00	90.00
August	30.66	21.50	91.00
September	31.50	18.80	86.00
October	33.16	22.00	52.00
November	28.83	19.00	36.00
December	26.66	12.33	41.00
January	21.83	9.80	44.00
February	28.33	13.83	38.00

Results and Discussion

The incidence of flat/poorly freezable semen ejaculates in Mehsana buffalo bulls was found to be 10.57% while the same for Murrah buffalo bulls was 11.03%. Overall incidence for both the breeds was 10.79%. There were significant differences between bulls and between breeds ($P < 0.05$) for the incidence of such ejaculates. From Table 2, it can be seen that none of the ejaculates from Mehsana buffalo bull MH-2, had to be discarded due to non-motility or poor freezability. Similarly, Mehsana buffalo bulls-MH-1, MH-6, MH-4 and Murrah buffalo bulls-MR-2, MR-1 & MR-3 had low incidence of occurrence of flat or poorly freezable semen ejaculates compared to other bulls. Three out of 7 (42.85%) Mehsana buffalo bulls & 5 out of 8 (62.5%) Murrah buffalo bulls had a discard rate above 10%. This may be due to older age of Murrah compared to the Mehsana buffalo bulls. It can be seen from Figure-1 that incidence of flat/poorly freezable ejaculates was high during Cold months of January & February (14.10 & 24.67%). Incidence was nil during the month of November. Peaks occurred during March, June & October months (13.95%, 13.04% & 14.10). Lowest incidence of flat/poorly freezable ejaculates was associated with elevation in mean post-thaw recovery percentage as well as in conception rates (Figure-1).

Abhi *et al.* (1968) found that 74 semen ejaculates out of 280 were initially non-motile in 4 Murrah buffalo bulls. Incidence was more during extreme weather conditions of summer & winter. They also found that quality of such ejaculates was poorer to normal ejaculates. Tomar & Misra (1971) studied physical & biochemical characteristics of 73 non-motile & 214 progressively motile Murrah buffalo semen ejaculates and found that non-motile ejaculates had significantly low values for ejaculate volume, live sperm per cent, sperm concentration, cold shock resistance, keeping quality and fructose content compared to normal ejaculates. Nema *et al.* (1983) observed that 38 of the 100 Surti buffalo semen ejaculates were initially nonmotile. They also found that these ejaculates were poor in physical and

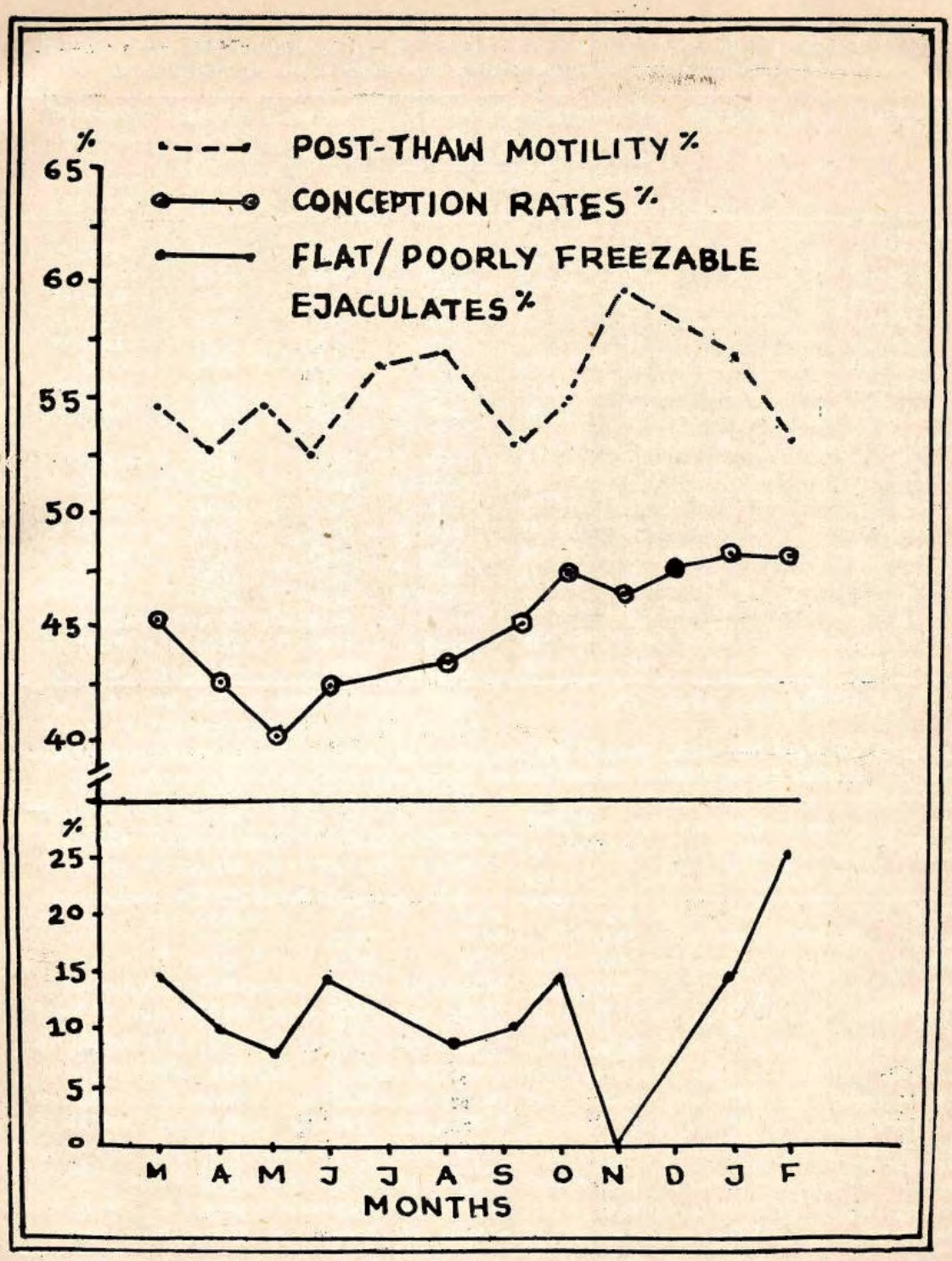

Fig. 1: Incidence of flat/poorly freezable semen—ejaculates (Mehsana and Murrah) and its relation with post—thaw motility and conception rates.

TABLE 2: Incidence of flat/poorly freezable semen ejaculates and its relation with mean post-thaw motility & losses in motility during freezing in Mehsana & Murrah buffalo bulls.

Breed & Buffalo bull	Semen ejaculates studied	Flat/Poorly freezable ejaculates		Mean post-thaw motility % (A)	% loss during freezing in motility (B)	Correlation coefficient (r) between (A) & (B)
		No.	%			
Mehsana						
MH — 1	83	1	1.20	57.17	19.91	—0.8938*
MH — 2	86	0	0.00	62.08	17.92	—0.9032**
MH — 3	63	21	33.33	47.19	28.13	—0.9613**
MH — 4	76	5	6.57	55.42	20.83	—0.9115**
MH — 5	49	5	10.20	56.25	21.25	—0.8666**
MH — 6	65	1	1.54	63.54	16.04	—0.6482*
MH — 7	51	17	33.33	52.08	22.92	—0.9434**
Overall for breed	473	50	10.57	56.26	21.00	—0.9240**
Murrah						
MR — 1	50	2	4.00	57.92	19.58	—0.8846**
MR — 2	89	2	2.25	58.77	18.18	—0.8112**
MR — 3	43	2	4.65	52.50	25.42	—0.8372**
MR — 4	36	11	30.56	50.25	25.58	—0.8430**
MR — 5	32	4	12.50	51.25	26.08	—0.9156**
MR — 6	63	12	19.05	54.79	21.88	—0.9341**
MR — 7	54	7	12.96	51.67	26.66	—0.9595**
MR — 8	68	8	11.76	54.58	22.71	—0.8848**
Overall for breed	435	48	11.03	53.97	23.26	—0.9281
Overall both breeds	908	98	10.79	55.12	22.13	—

* $P < 0.05$ ** $P < 0.01$

TABLE 3: Mean per cent initial motility, per cent losses in motility during freezing & post-thaw motility during different months/seasons in Mehsana & Murrah buffalo bulls.

Month/Season	Mehsana			Murrah		
	Initial motility	Loss in motility during freezing	Post thaw motility	Initial motility	Loss in motility during freezing	Post thaw motility
March	75.71	22.86	52.86	77.76	22.19	55.63
April	75.00	21.43	53.57	76.25	23.75	52.50
May	78.57	24.64	53.93	76.25	22.19	54.38
June	76.43	23.21	53.21	77.66	25.78	51.88
Hot Season	**76.44**	**23.04**	**53.39**	**77.06**	**23.48**	**53.60**
July	77.86	19.19	58.57	77.38	24.67	52.71
August	77.14	20.36	56.79	77.40	22.52	54.88
September	77.14	19.29	57.86	76.25	26.25	50.00
October	79.29	20.71	58.57	75.63	24.69	50.93
Wet Season	**77.86**	**19.19**	**57.95**	**76.67**	**24.53**	**52.13**
November	77.87	15.71	62.14	79.38	21.25	58.13
December	78.57	20.00	58.57	76.75	19.25	57.50
January	77.14	19.64	57.50	78.75	23.44	55.31
February	76.43	24.28	52.14	77.13	21.56	55.63
Cold Season	**77.50**	**19.91**	**57.58**	**78.00**	**21.38**	**56.64**
Overall	77.35	20.95	56.31	77.23	23.13	54.12

biochemical characteristics compared to normal ejaculates. The findings of present study corroborates with observations of Abhi *et al.* (1968) that incidence of such ejaculates is more during extreme cold & extreme hot weather.

Mean post-thaw motility % and losses during freezing and correlation coefficients between these 2 characters are given in Table 2. The differences between different buffalo bulls and between 2 breeds were statistically significant ($P < 0.05$). It was observed that the buffalo bulls in which the mean losses during freezing were high, had usually higher incidence of flat/poorly freezable semen ejaculates as well as lower mean post-thaw motility scores compared to other buffalo bulls (e.g. MH_3 & MH_7 buffalo bulls in Mehsana breed & MR-4 buffalo bull in Murrah breed).

Mean post-thaw motility was highest in Mehsana buffalo bull MH-6 (63·54) which had a lowest incidence of initially non-motile/poorly freezable semen ejaculates. Post-thaw recovery in Mehsana buffalo bull MH-3 was below 50% while it was above 50 % in rest of the buffalo bulls. Highest post-thaw motility was observed during the month of November (62.14 %, Figure-1) and during the cooler months of Wet & Cold seasons (57.95 % & 57.58 %, respectively). During the Hot season the mean post-thaw motility was 53.39 % which was lower than other seasons (Table-3).

Loss of motile spermatozoa during freezing were highest during the month of June (25.78 %) and during the Hot season (23.48 %) compared to other months and seasons (Table 3). A highly significant negative correlation was found between the post-thaw motility percentage and percentage loss in motility during freeze-thaw procedure in almost all the buffalo bulls (Table 2). It ranged from -0.6482^{*} to -0.9613^{**} in different buffalo bulls of Mehsana and Murrah breeds.

Jani (1982) observed that 43.5 % 39-2% & 17.3 % of 20 Surti buffalo bulls and 3 Holstein Friesian cow bulls donated semen which had satisfactory, moderately suitable and unsuitable freezability respectively. He observed the mean post-thaw motility in Tris-fructose-egg yolk-glycerol dilutor at 0 & 48 hour to be 61.00 & 56.60 %, respectively in Surti buffalo semen. Mean post-haw motility in the present study ranged between 52.14 to 62.14 % in Mehsana buffalo semen and between 50.00 to 58.13 % in Murrah buffalo semen (Table-3). Reddy *et al.* (1982) reported the prefreeze and postfreeze motility of 79.56 % and 51.91 % in Surti buffalo bulls which is comparable to findings of the present study. Tuli *et al.* (1984) have observed the initial motility and post-thaw motility in summer and winter months as 64.64 ± 1.37 & 66.25 ± 1.14 % and 41.78 ± 1.45 & 49.64 ± 1.18 % respectively. They found that the loss in motility between equilibration and thawing was 14 % during summer while it was 8 % during the winter season. In the present study loss in motile spermatozoa has been compared between initial motility and post-thaw motility (Table 3).

Abhi (1982) reported that the average initial motility of the semen ejaculates from 8 Murrah buffalo bulls was 63.3 % while post-thaw motility obtained after freezing with Tris-dilutor was 48.0 %. Semen from 3 buffalo bulls was not suitable for freezing & had to be rejected. These findings are comparable to the observations made in the present study.

Conclusions

It is concluded that the semen from all the valuable and high pedigreed Mehsana & Murrah buffalo bulls is not equally freezable. Spermatozoan losses are more during freeze-thaw procedures and vary significantly between bulls, breeds, months and seasons. Spermatozoan losses are more during extreme hot and extreme cold period of the year.

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The authors are thankful to Dr. D. V. Mokashi, Director of Animal Husbandry, Gujarat State, Dr. M. R. Patel, Principal, Gujarat Veterinary College, Anand and Dr. J. M. Vyas, Project Officer, Intensive Cattle Development Programme, Mehsana for their encouragement and for providing facilities to undertake the present study.

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IJAR 7: 1: 7-14, 1986

Seminal Characters, Freezability And Fertility In Mehsana & Murrah Buffalo Bulls.

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ABSTRACT

Eighty four semen collections from seven healthy Mehsana and ninety six semen collections from eight Murrah buffalo bulls were studied for a period of one year. Mean ejaculate volume (ml) initial motility (%), sperm concentration/ml ($\times 10^6$), sperm concentration/ ejaculate ($\times 10^6$), dead & abnormal spermatozoa (%) were: 3.98 ± 0.35, 77.26 ± 0.53, 1334.75 ± 16.30, 5302.02 ± 311.10, 6.82 ± 0.01, 9.68 ± 0.07 and 8.33 ± 0.25, respectively in Mehsana buffalo bulls. These values for Murrah buffalo bulls were 3.84 ± 0.17, 77.23 ± 0.24, 1341.12 ± 19.88, 5312.18 ± 154.40, 6.83 ± 0.02, 9.58 ± 0.16 and 7.55 ± 0.17, respectively. Semen quality was comparatively poor during hot months of summer and it improved as rainy season advanced with fall in ambient temperatures.

Mean post-thaw motility (%), losses during freezing (%) and conception rates (%) for Mehsana buffalo semen were: 56.26 ± 5.45, 21.0 ± 1.47 and 44.82 ± 0.38 per cent respectively. These values for Murrah buffalo semen were: 53.97 ± 1.08, 23.26 ± 1.13 and 44.69 ± 0.77 per cent respectively. Freezability and conception rates were found to be superior during the cooler months from September to February in both the breeds.

Highly significant positive correlation of 0.6124 for Mehsana buffalo semen and 0.3042 for Murrah buffalo semen was observed between initial motility and post thaw motility of semen. Highly significant negative correlation was found between conception rate and ambient temperatures in both the breeds.

Number of inseminations done, conception rates and number of semen doses prepared per buffalo bull using chilled and frozen semen techniques has been compared.

* * *

Recent advances in artificial breeding of cattle in developed countries, primarily can be attributed to wide spread application of frozen semen technique. It is one of the most sophisticated techniques of genetic improvement of cattle and buffaloes. Last decade has seen rapid strides being made in deep freezing of buffalo semen. Mehsana buffaloes being an important breed of buffaloes of Gujarat State, present study has been undertaken to investigate seminal characteristics, freezability and fertility of Mehsana and Murrah buffalo bulls.

1. Officer in charge, Central Semen collection Station, Mehsana.
2. Ex. Project Officer, I.C.D.P., Mehsana.

TABLE 1: Climatic components during different seasons (Means).

Season	Ambient temperature (°C) Maximum	Minimum	Relative humidity (%)
Hot season (Mar.-June)	39.69	26.42	50.80
Wet season (July-Sept.)	32.15	21.33	80.50
Cold season (Oct.-Feb.)	26.41	13.74	30.30

Materials and Methods

Eighty four semen collections from seven healthy Mehsana and Ninety six semen collections from eight Murrah buffalo bulls were studied for a period of one year (April, 84 to March, 85). Buffalo bulls were located at Central Semen Collection Station, Mehsana and were on a collection schedule of 2-3 ejaculates per week. They were under identical conditions of management and nutrition. To alleviate the heat stress during the hot summer months, they were given cool shed and twice daily cold water baths.

After collecting semen with artificial vagina, it was immediately examined for macroscopic and microscopic characteristics in the laboratory as per Herman and Madden (1953). Sperm concentration and pH were determined with photoelectric colorimeter and pH meter respectively. The semen ejaculates having initial motility over 70 percent were diluted in Tris-fructose-egg yok glycerol dilutor and frozen as described by Vasanth (1979) after equilibration for 4-5 hours at 5°C. The post thaw motility was recorded within half an hour after freezing. Conception rates were obtained from field inseminations carried out and followed for pregnancy diagnosis by the technical field staff of the Intensive Cattle Development Programme, Mehsana for the frozen semen doses supplied during the whole year. Conception rates were calculated as per Nair (1975) using the following formula.

$$C.R.\% = \frac{\text{No. of animals found pregnant}}{\text{No. of animals inseminated \& followed for pregnancy}} \times 100.$$

Data on conception rates for liquid semen and frozen semen and doses produced per buffalo bull were obtained for comparison from ICDP, Mehsana records. Statistical analysis was carried out as per Snedecor & Cochran (1971) on Spactrum-7 computer belonging to the Department of Agricultural Statistics, B.A. College of Agriculture, Anand. Climatological data during the year under study has been presented in Table 1.

Results and Discussion

Seasonal variations in seminal characteristics of Mehsana and Murrah buffalo bulls has been given in Table 2 & 3 respectively. It can be seen that variations between seasons were significant ($P<0.05$) for sperm concentration/ml in both the breeds; sperm concentration/ ejaculate in Mehsana buffalo bulls and abnormal spermatozoa percentage in Murrah buffalo bulls. Mean seminal characters for Mehsana buffalo bulls were: volume (ml) 3.98 ± 0.35, initial motility (%) 77.26 ± 0.53, sperm concentration/ml ($\times10^6$) 1334.75 ± 16.30, sperm concentration/ejaculate ($\times10^6$) 5302.02 ± 311.10, pH 6.82 ± 0.01, dead sper-

TAELE 2: Semen characters of Mehsana buffalo bulls during different seasons.

Season	Volume (ml)	Intial motility %	Sperm conc/ ml × 10^6	Sperm conc/ eja. × 10^6	pH	Dead sperm %	Abnormal sperm %
Hot	3.76	76.44	1234.78	4478.94	6.81	9.90	9.43
	±0.32	±0.52	±17.80	±321.11	±0.01	±0.08	±0.25
Wet	4.30	77.86	1348.61	5760.53*	6.84	9.48	7.85
	±0.43	± 0.64	±20.22	±298.17	±0.02	±0.09	±0.28
Cold	3.90	77.50	1420.93*	5666.59	6.82	9.66	7.73
	±0.29	±0.48	±15.70	±301.80	±0.01	±0.07	±0.22
Over all	3.98	77.26	1334.75	5302.02	6.82	9.68	8.33
	±0.35	±0.53	±16.30	±311.10	±0.01	±0.07	±0.25

* P < 0.05

TABLE 3: Semen characters of Murrah buffalo bulls during different seasons.

Season	Volume (ml)	Initial motility %	Sperm conc/ ml × 10^6	Sperm conc/ eja. × 10^6	pH	Dead sperm %	Abnormal sperm %
Hot	3.56	77.02	1237.91	4882.53	6.82	9.72	8.90*
	±0.19	±0.33	±21.25	±21.25	±0.01	±0.11	±0.12
Wet	4.09	76.67	1386.23	5638.35	6.84	9.77	7.02
	±0.23	±0.17	±17.25	±188.92	±0.02	±0.08	±0.24
Cold	3.85	78.00	1399.22*	5445.66	6.83	9.25	6.79
	±0.14	±0.23	±19.23	±132.77	±0.01	±0.08	±0.15
Over all	3.84	77.23	1341.12	5312.18	6.83	9.58	7.55
	±0.17	±0.24	±19.88	±154.40	±0.02	±0.16	±0.17

* P < 0.05

matozoa (%) 9.68±0.07 and abnormal spermatozoa (%) 8.33±0.25. These values for Murrah buffalo bulls were: volume (ml) 3.84±0.17, initial motility (%) 77.23±0.24, sperm concentration/ml (×10^6) 1341.12±19.88, sperm concentration/ejaculate (×10^6) 5312.18 ±154.4, pH 6.83±0.02, dead spermatoeoa (%) 9.58±0.16 and abnormal spermatozoa (%) 7.55±0.17. Semen quality was superior during the cooler months of Wet and Cold seasons compared to Hot season. However, the decline in semen quality during the Hot months was not very marked probably due to the provision of cool sheds and cold water baths given to the buffalo bulls during the Hot summer months.

In case of Mehsana buffalo bulls the differences in semen characters between bulls and between months were statistically significant (P<0.05) for ejaculate volume, sperm concentration/ml, sperm concentration/ejaculate and between months for initial motility and abnormal spermatozoa. Whereas, these differences were significant (P<0.05) between bulls and between months for ejaculate volume, sperm concentration/ejaculate and between months for hydrogen ion concentration in Murrah buffalo bulls. Semen quality improved as rainy season advanced and ambient temperature became cooler. Difference in semen characters between breeds were non significant.

Sengupta *et al.* (1963) reported that the

FIGURE:1.

POST-THAW MOTILITY AND CONCEPTION RATES IN RELATION TO AMBIENT TEMPERATURES IN MEHSANA/MURRAH BUFFALO BULLS.

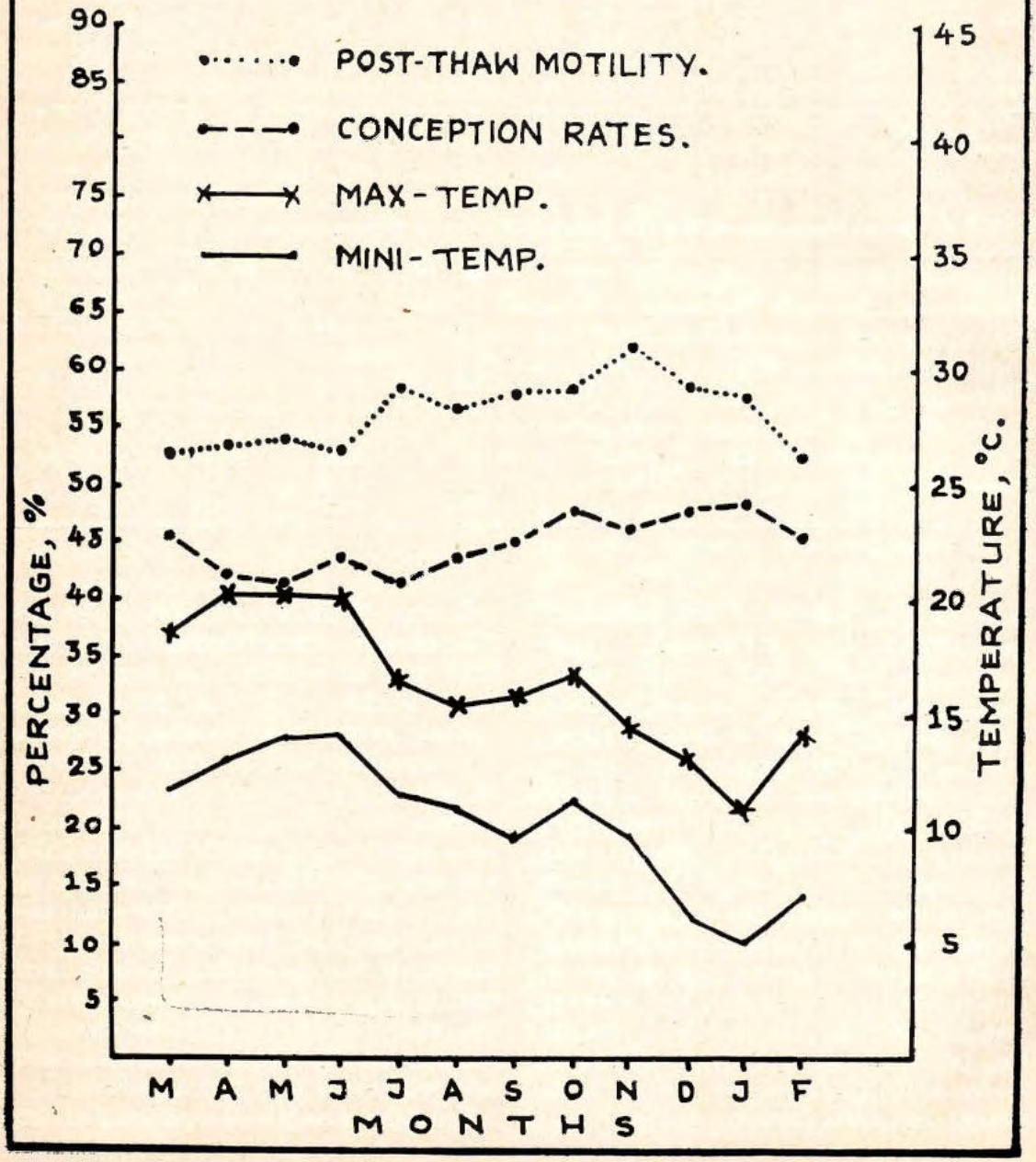


TABLE 4: Post thaw motility, loss in motility during freezing and conception rates during different seasons in Mehsana and Murrah buffalo bulls.

Season	Post-thaw motility %		Loss in motility during freezing %		Conception rates %	
	Mehsana	Murrah	Mehsana	Murrah	Mehsana	Murrah
Hot	54.57 ± 5.21	53.24 ± 0.98	23.14 ± 1.61 [30.25']	23.48 ± 1.06 [30.48']	43.29 ± 0.28 (4271)	42.40 ± 0.59 (5743)
Wet	57.95 ± 3.11	52.13 ± 1.11	19.91 ± 1.32 [25.37']	24.53 ± 1.39 [31.90']	44.60 ± 0.30 (12662)	45.02 ± 0.68 (9928)
Cold	57.58 ± 5.75	56.64 ± 1.13	19.94 ± 1.29 [26.37']	21.77 ± 1.14 [27.97']	46.57* ± 0.49 (11996)	46.65* ± 0.97 (8524)
Over all	56.26 ± 5.45	53.97 ± 1.08	21.00 ± 1.47 [27.39']	23.26 ± 1.13 [30.12']	44.82 ± 0.38 (28929)	44.69 ± 0.77 (24195)

* $P < 0.05$

Figures in brackets give loss in motile spermatozoa relative to initial motile spermatozoa.
Figures in parenthesis indicate number of inseminations followed for pregnancy.

TABLE 5: Correlation coefficients between semen characteristics, climatic components and conception rates in Mehsana and Murrah buffalo bulls.

Relationship between	Correlation coefficient (r)	
	Mehsana	Murrah
—Initial motility and abnormal sperm %	—0.1747^{ns}	—0.2053*
—Ejaculate volume and sperm conc/ejaculate	+0.7957**	+0.8651**
—Initial motility and post thaw motility	+0.6124**	+0.3042**
—Initial motility and loss in motility during freezing	—0.2636*	—0.0923^{ns}
—Sperm conc/ml and abnormal sperm %	—0.3366**	—0.2400*
—Sperm conc/ml and maximum temperature	—0.4803**	—0.2186*
—Sperm conc/ml and minimum temperature	—0.4299**	—0.1146^{ns}
—Abnormal sperm and post-thaw motility	—0.3276**	—0.1100^{ns}
—Conception rate and dead sperm %	—0.2313*	—0.0879^{ns}
—Conception rate and maximum temperature	—9.330**	—0.4607**
—Conception rate and minimum temperature	—0.3508**	—0.4521**

* = $P < 0.05$.
** = $P < 0.01$.
NS = Nonsignificant.

semen quality was worst during the summer months and improved with onset of rainy season. Gopalkrishna *et al.* (1978) observed the volume, initial motility, live percentage and sperm head abnormalities to vary significantly between months in Murrah buffalo bulls. Kapoor (1973) recorded the best semen quality in Murrah buffalo bulls during the months of August to December. Observations of these workers on semen characteristics were comparable to present

findings.

Mean post-thaw motility, loss in motility during freeze-thaw procedures and conception rates for different seasons are given for Mehsana and Murrah buffalo bulls in Table 4. The post-thaw motility was higher during the Wet and Cold seasons compared to Hot season, however the diffdrences were statistically non—significant. Spermatozoan losses relative to initial motility were higher during Hot season in Mehsana (30.25%) and during Hot & Wet seasons in Murrah (30.48 & 31.90%) buffalo semen. The conception rates based on a total of 28929 and 24195 inseminations, followed for pregnancy for Mehsana and Murrah buffalo bulls were significantly ($P<0.05$) higher during the cold season compared to other seasons. They were comparatively lower during the Hot season for both the breeds. The relationship between post-thaw motility, conception rates and ambient temperatures has been given in Figure 1. The decline of maximum and minimum ambient temperature is associated with rise in post-thaw motility and conception rates of Mehsana and Mnrrah buffalo Semen. Buffalo bulls varied significantly ($P<0.05$) from each other for post-thaw motility in both the breeds. Higher post-thaw motility was not always associated with higher conception rates in these buffalo bulls.

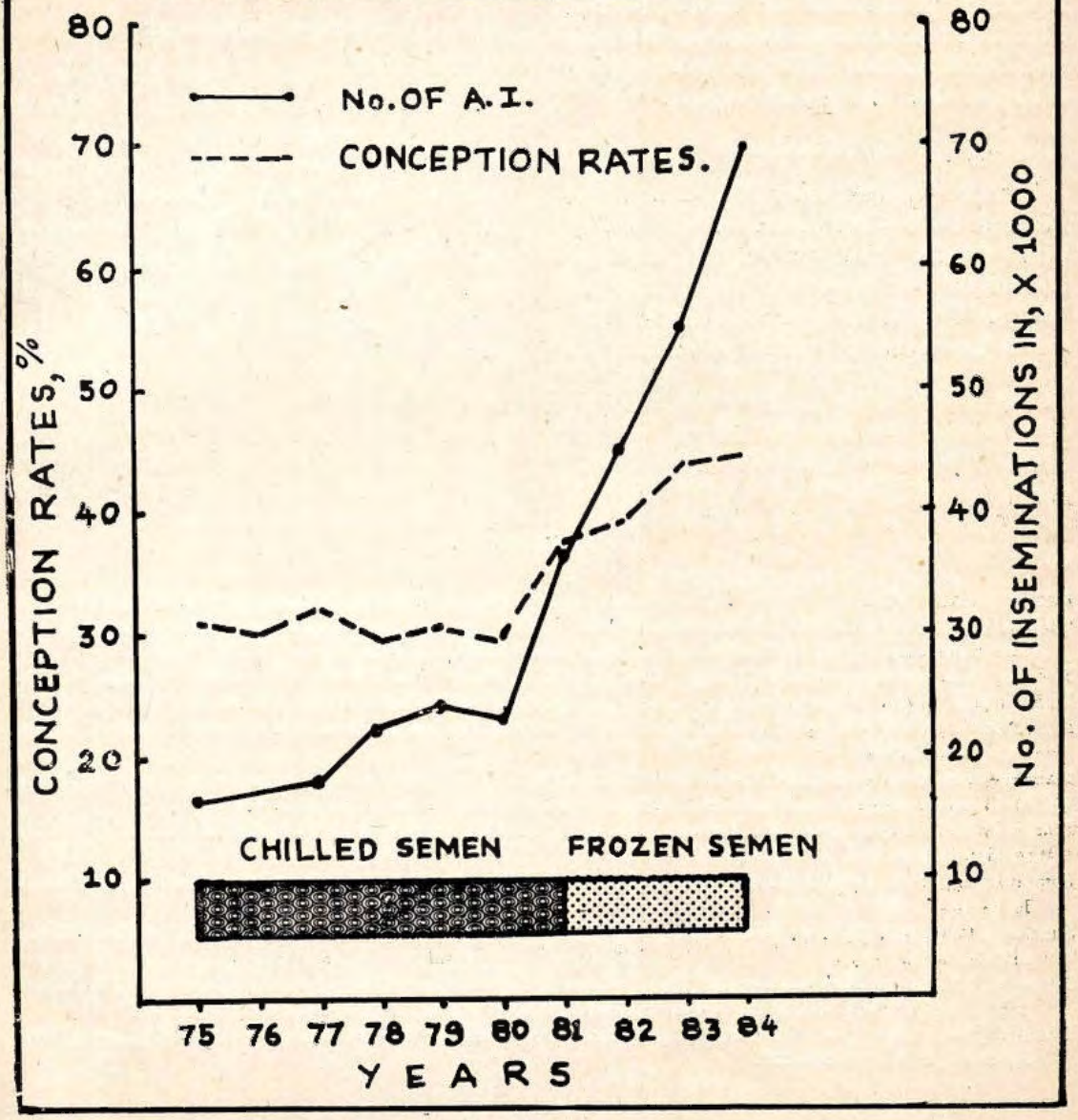
Mean post-thaw motility and conception rates for Mehsana buffalo bull semen were 56.26 ± 5.45 and 44.82 ± 0.38 percents while these values for Murrah buffalo semen were 53.97 ± 1.08 and 44.69 ± 0.77. The differences between the breeds were statistically non—significant. Vasanth (1978) has reported conception rate of 45% based on 13659 inseminations using Surti and Murrah buffalo frozen semen in a field study. Reddy *et al.* (1982) reported a mean post-thaw motility of 51.91% and conception rate of 47% based on 11859 inseminations. Roychaudhari (1978) has reported post-thaw motility and conception rate of 50% and 41% respectively for Surti buffalo semen. Radhakrishna *et al.* (1983) have repoi ted the conception rates with frozen semen to vary from 40.00 percent to 52.53 percent between the years 1977-78 to 1981-82 with overall mean of 48.61 percent in field studies involving over 35292 buffalo inseminations, which were followed for pregnancy. The post-thaw motility and conception rates observed in the present study are comparable to findings of these workers.

The correlation coefficients between seminal characters, climatic components and conception rates have been presented in Table 5. It can be seen that there is a highly significant ($P<0.01$), positive correlationship ($r= +0.6124$ for Mehsana and $r = + 0.3042$ for Murrah buffalo bulls) between initial motility and post-thaw motility of semen, and a highly significant negative correlationship between conception rates and maximum & minimum ambient temperatures.

Figure 2 gives a comparison between chilled and frozen semen techniques with respects to number of inseminations and conception rates. It was seen that the number of inseminations performed and conception rates as well as number of semen doses produced per buffalo bull have increased due to adoption of frozen semen technique compared to chilled semen. The number of artificial insemination performed and conception rates which ranged from 16268 to 23174 and 29.18 to 31.80% during the years 1975-81, when chilled semen was used, increased to 36102 to 69328 inseminations and 37.50 to 44.91%, respectively—with the introduction of frozen semen

FIGURE: 2.

NUMBER OF INSEMINATIONS AND CONCEPTION RATES USING CHILLED AND FROZEN MEHSANA/ MURRAH BUFFALO SEMEN.



technique. The chilled semen doses produced per buffalo bull ranged between 3151.54 to 3801.62, whereas 5111.42 to 6881.12 frozen semen doses per buffalo bull could be produced indicating better utilization of the superior germ plasm with frozen semen technique. Gupta *et al.* (1981) have reported production of 12908.16 and 11115.72 frozen semen doses per Murrah and Surti buffalo bull per year which is higher than the present report.

Conclusions

Semen characteristics of Mehsana and Murrah buffalo bulls showed comparable and parallel changes during different seasons of the year. Cooler months of Wet and Cold seasons were favourable for better quality semen production in these animals. Post-thaw motility differed significantly between buffalo bulls and between months. Artificial inseminations, conception rates and semen doses produced per buffalo bull have increased with advent of frozen semen technique in comparison with chilled semen technique.

Acknowledgement

The authors express their gratitude to Dr. D.V. Mokashi, Director of Animal Husbandry, Gujarat State, Dr. M.R. Patel, Dean, College of Veterinary Science & Animal Husbandry, Anand and Dr. J.M. Vyas, Project Officer, Intensive Cattle Development Programme, Mehsana for their encouragement and for providing facilities to undertake the present study.

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Cryopreservation Of Buffalo Semen In Modified Tris Extender Containing Lactose Or Sucrose With Various Levels Of Glycerol

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ABSTRACT

Cryopreservation of Surti buffalo semen in modified tris lactose and tris sucrose extenders with different level of glycerol is reported. Addition of 73 mM of lactose or sucrose to tris extender maintaining the osmolarity at 381 mM was beneficiary in cryopreservation of buffalo semen in 0.5 ml French straws. In tris lactose extender with 3 or 6 per cent glycerol and tris sucrose extender with 6 per cent glycerol the post thaw motility was higher than tne control (tris extender).

* * *

In cryopreservation of bovine semen one of the factors that influence is type, level and presence of different cryoprotective agents in the semen extenders. Glycerol has been extensively used as a cryoprotective agent for bull semen (Erikson *et al.*, 1954; Martin, 1965; Pickett and Berndtson, 1974). However, a large portion of the spermatozoa are rendered immotile despite tne presence of glycerol (Unal *et al.*, 1978). Certain sugars also provide cryoprotection for bovine spermatozoa (Nagase *et al.*, 1964; Berndtson and Foote, 1972). Direct comparison of glycerol and sugars as cryoprotective agents for freezing of white cattle semen are avilable (Unal *et al.*, 1978), whereas it is lacking in buffaloes. In the present study an attempt has been made to incoiporate lactose or sucrose as cryoprotective agent in tris extender with different levels of glycerol for freezing of Surti buffalo semen.

Materials and Methods

Semen samples were collected from 5 healthy bulls at 3 days interval. Preliminary tests like color, consistency, volume, mas activity and sperm concentration were done to eliminate substandard semen. Fifteen ejaculates were subjected for present study.

The composition of the experimental extenders was as follows:

Composition of stock buffer	*Tris lactose*	*Tris sucrose*
Tris (hydroxymethyl) amino methane	2.271 gm	2.271 gm
Citric acid	1.276 gm	1.276 gm
Fructose	1.000 gm	1.000 gm
Lactose	2.630 gm	—
Water	100.000 ml	100.000 ml
Sucrose	—	2.500 gm

Extender composition

Egg yolk 20 per cent
Glycerol 0, 1.5, 3.0 or 6.0 per cent
Stock buffer qs to 100 ml to make respective extenders

Tris citric acid fructose egg yolk glycerol extender (Vasanth, 1978) was taken as control. Split semen samples were diluted in different extenders at 35°C such that each 0.5 ml French straw contained 30×10^6 motile sperms. Straws were filled with semen at room temperature and sealed using polyvinyle alcohol (PVA) powder. Identification of straws with different extenders was done utilizing different color combination of straw and PVA powder. After 4 hours of equilibriation period straws were frozen over liquid nitrogen vapour for 10 minutes and then plunged into liquid nitrogen. The straws were collected from freezer and stored in frozen semen container. Initial motility (immediately after extension of semen), prefreeze motility (after 4 hours of equilibriation) and post thaw motility (two to four hours after freezing) were recorded. Any difficulty arising from granularity of the diluent when examining the samples for motility was overcome by adding 0.5 ml of tri sodium citrate solution (3 per cent) adjusted to pH 7 with citric acid to semen samples as described by Floulkes *et al.* (1977). The percentage values of motility were transformed to arcsine values and subjected for analysis of variance. The mean comparison was done by Students "t" test (Snedecor and Cochran, 1967).

Results

The mean initial, pre-freeze and post-thaw motility is reported in Table 1.

In modified tris lactose extender with 0, 1.5, 3.0 and 6.0 per cent glycerol the initial and prefreeze motility was lower than the control whereas with 3 and 6 per cent glycerol the post thaw motility was better than the control. The variations in motility between different levels of glycerol and the control were not significant except in post thaw motility in tris lactose extender without glycerol.

In tris sucrose extender with varying levels of glycerol the initial and prefreeze motility was lower than the control, whereas with 6 per cent glycerol level the post thaw motility was better than the control. Variations in motility between different levels of glycerol and the control were not significant except for the post thaw motility in tris sucrose

TABLE 1: The mean percentage values of initial, pre freeze and post-thaw motility in Tris-Lactose and Tris-Sucrose extenders.

Extender	Initial motility	Pre-freeze motility	Post-thaw motility
Tris-Lactose extender without glycerol	$74.00^{w} \pm 1.56$	$69.33^{w} \pm 1.94$	$6.66^{w} \pm 1.05$
Tris-lactose extender with 1.5% glycerol	$73.66^{w} \pm 1.50$	$68.00^{w} \pm 1.94$	$27.33^{x} \pm 4.25$
Tris-lactose extender with 3.0% glycerol	$72.66^{w} \pm 1.37$	$67.33^{w} \pm 1.88$	$41.00^{y} \pm 3.45$
Tris-lactose extender with 6% glycerol	$72.33^{w} \pm 1.37$	$67.00^{w} \pm 1.60$	$37.26^{y} \pm 3.59$
Tris-sucrose extender without glycerol	$73.00^{a} \pm 1.45$	$68.00^{a} \pm 1.81$	$9.00^{a} \pm 1.21$
Tris-sucrose extender with 1.5% glycerol	$72.33^{a} \pm 1.37$	$67.33^{a} \pm 1.89$	$25.66^{b} \pm 3.84$
Tris-sucrose extender with 3% glycerol	$72.00^{a} \pm 1.36$	$66.00^{a} \pm 2.19$	$33.66^{bc} \pm 4.01$
Tris-sucrose extender with 6% glycerol	$71.33^{a} \pm 1.50$	$65.66^{a} \pm 2.17$	$37.33^{c} \pm 3.65$
Tris extender (control)	$74.66^{aw} \pm 1.24$	$69.33^{aw} \pm 1.75$	$35.33^{cxy} \pm 2.82$

Between extenders, means with common superscript do not vary significantly at $p < 0.05$.

extender with 0 and 1.5 per cent glycerol level.

Discussion

Bull spermatozoa do not metabolise di and tri sacchazides in presence of monosaccharides and they are added as cryopreservatives in semen extenders for freezing. The large sugar molecules are believed to maintain osmotic balance by acting as substitutes for electrolytes in semen extenders. The mode of cryoprotection afforded by large sugar molecules is different from that of glycerol. These sugars are some times complimentary and useful in presence of glycerol which is necessary for freezing of bull spermatozoa (Salisbury *et al.*, 1978). Lactose and sucrose which are disaccharides would probably act in a similar way when incorporated in tris extender containing glycerol for buffalo semen freezing. For buffalo semen freezing in pellets 7 per cent glycerol was found to be better then 14 per cent glycerol with egg yolk citrate diluent frozen to — 79°C (Rathore, 1965).

Milk sodium citrate extender with 7 per cent glycerol gave better post thaw motility than 5 or 9 per cent glycerol when buffalo semen was frozen in ampoules using dry ice alcohol bath (Pavithran *et al.*, 1972). In the present study 3 and 6 per cent glycerol in the modified tris lactose extender and 6 per cent glycerol in modified tris sucrose extender gave better post thaw motility then the control (Table 1) indicating that addition of lactose or sucrose into tris extender is beneficiary in cryopreservation of buffalo semen in 0.5 ml French straws.

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Superovulatory Response Of Cross-bred Cows and Heifers To Pregnant Mare Serum Gonadotrophin

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ABSTRACT

The superovulatory response of ten cows and five heifers to 1500-2000 I.U. of PMSG given in follicular and luteal phases of oestrous cycle was recorded. Animals in luteal phase stimulation received 0.5 mg cloprostenol 48 to 72 hours after PMSG injection. Visualisation of the ovaries was accomplished by cystoscope passed through a Jarrett's sheep rumen cannula fixed in the right paralumbar fossa.

The mean ovulatory rates were 2.50 and 6.33 for follicular phase stimulations with 1500 and 2000 I.U. PMSG respectively. Luteal phase stimulations yielded a mean ovulatory rate of 5.80 and 17.67 with 1500 and 2000 I.U. of PMSG respectively.

Luteal phase stimulations yielded greater number of ovulations than the follicular phase stimulation. The interval between gonadotrophin injection and subsequent oestrus was 3-4 days in follicular phase and 5-6 days in luteal phase stimulations.

* * *

Chang (1949) and Hammond (1950) suggested the use of exogenous hormones for the production of large number of fertilized ova to make embryo transfer feasible in cattle. Dowling (1949), Umbaugh (1949) and Rowson (1951) performed the initial experiments. Gordon *et al.* (1962) described pregnant mare serum (PMSG) as containing both the follicular stimulating hormone and luteinizing hormone activities. Hammond and Bhattacharya (1944), and Dowling (1949) reported on the use of PMSG. Rowson (1951) compared the ovarian response to the processed and whole PMSG. PMSG in the form of a purified freeze dried powder is being used extensively by most research centres in the world (Newcomb and Rowson, 1976). The present study was undertaken to evaluate the superovulatory response of cross-bred cows to different doses of PMSG during follicular phase and luteal phase of the cycle.

Materials and Methods

Experimental animals:

The experiments were conducted on ten pluriparous cows and five heifers. The cows included five Holstein-cross and five Jersey cross. The five heifers were Jersey-Friesian-others cross. All the animals were confined to individual stalls during the period of experimentation. They were fed on concentrates, green grass and paddy straw. The animals were found to have normal reproductive organs on rectal palpation. They were cycling normally and some cows were lactating at the time of receiving the hormones.

Visualisat ion ofOvaries:

In order to visualise the ovaries, a Jarrett's sheep ruminal cannula was fixed in the right paralumbar fossa of all the animals. The cannula was sterilized by immersion in 0.5% cetrimide* for 30 min and then washed with sterile normal saline prior to use. Following aseptic preparation of the site, anaesthesia was accomplished by "<" block technique with 20 ml of 2% lignocain hydrochloride solution. A 6 cm long cutaneous incision was made, and by blunt dissection the abdominal muscles were separated. Peritoneum was incised and the previously sterilized cannula was inserted into the abdominal cavity in such a fashion that one end was against the internal abdominal wall and the other end protruding out of the surgical wound. The laparotomy wound was sutured around the cannula. The outside opening of the cannula was closed with a screw cap.

The ovaries were visualised by removing the screw cap and introducing a sterilized cystoscope through the cannula. The cystoscope was sterilized in the same manner as the cannula. The ovaries were examined in situ. Sometimes the ovaries were grasped per rectum and directed towards the cystoscope. Following completion of the ovarian examination, the cystoscope was removed and the screw cap replaced.

Hormonal treatments:

Two consecutive oestrous periods were observed and then only the normal treatment were given. Pregnant mare serum gonadotrophin** was used at a dose rate of 1500 to 2000 International Units in order to stimulate the ovaries of each animal, either in follicular or luteal phase of the oestrous cycle. The superovulatory drug was given as a simple intramuscular injection. Cloprostenol*** was given intramuscularly at the rate of 0.5 mg in 2 ml distilled water per cow to cows stimulated in luteal phase of the cycle.

Follicular phase stimulations:

Pregnant mare serum gonadotrophin was injected on day 16 of the oestrous cycle. Three cows and one heifer were treated with 1500 I.U. Two cows and one heifer were given 2000 I.U. of PMSG. The animals were observed for oestrous following PMSG injection. They were not given any further hormonal treatments.

Luteal phase stimulations:

Pregnant mare srum gonadotrophin was injected on day 10 of the oestrous cycle. Three cows and two heifers were injected with 1500 I.U. Two cows and one heifer received 2000 I.U. of PMSG. Forty eight to 72 hours after PMSG injection, cloprostenol was given to produce luteolysis.

Animals which were seen in standing oestrus were artificially inseminated. The insemination was done three times at 6 hourly intervals with frozen semen. The first insemination was performed 4 to 5 hours after observing the cow in standing oestrus.

The ovarian response was ascertained 5 to 6 days after oestrus by rectal palpation. These were confirmed by directly visualising the ovaries with the cystoscope through the cannula in the right paralumbar fossa.

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** Folligon, Inter Vet. International, Holland.
*** Estrumate, I.C.I. Great Britain.

TABLE 1: Ovarian response to follicular phase (day 16) stimulations with 1500-2000 I.U. PMSG in cows and heifers.

Animal	Age (Yrs)	PMSG dose (I.U.)	Oestrous following PMSG (days)	Day of ovarian examination following oestrus	Response of the Ovary					
					Right		Left		Total	
					Un-Ovulated follicles	Ovulations	Un-ovulated follicles	Ovulations	Un-Ovulated follicles	Ovulations
Cow	6	1500	3	6	1	0	1	1	2	1
Cow	4	1500	3	6	1	1	1	1	2	2
Cow	6	1500	4	5	2	0	1	1	3	1
Heifer	1	1500	3	5	2	0	2	2	7	6
Cow	6	2000	4	6	3	3	5	3	9	6
Cow	5	2000	3	5	4	2	3	2	7	4
Heifer	1.5	2000	4	6	3	5	3	4	6	9

TABLE 2: Ovarian response to luteal phase (day 10) stimulations with 1500-2000 I.U. PMSG and Cloprostenol in cows and heifers.

Animal	Age (Yrs)	PMSG dose (I.U.)	Cloprostenol dose (mg)	Oestrus following PMSG (days)	Day of ovarian examination following oestrus	Response of the ovary					
						Right		Left		Total	
						Un-ovulated follicles	Ovulated follicles	Un-ovulated follicles	Ovulated follicles	Un-ovulated follicles	Ovulated follicles
Cow	5	1500	0.5	5	6	1	1	1	3	2	4
Cow	6	1500	0.5	5	5	2	2	1	4	3	6
Cow	4	1500	0.5	6	6	1	2	1	4	2	6
Heifer	1	1500	0.5	5	6	1	3	2	4	3	7
Heifer	1.5	1500	0.5	5	5	2	2	1	4	3	6
Cow	6	2000	0.5	6	6	2	9	5	11	7	20
Cow	4	2000	0.5	5	5	3	7	3	10	6	17
Heifer	1	2000	0.5	6	6	4	7	2	9	6	16

Results and Discussion

Visualisation through the Jarrett cannula:

There was no reaction around the Jarrett cannula. Endoscopic examination was easy in viewing the right ovary. Manipulation of ovaries per rectum helped in visualisation of both the ovaries. Omentum invariably blocked the surface of the ovary, as reported by Megale *et al.* (1955), Dziuk *et al* (1958) and Lamond and Holmes (1965). These workers suggested restricting water and feed intake for 1 to 2 days prior to observation, keeping animal on a slope with hind quarters elevated, and directing ovaries towards the endoscope by rectal palpation.

Follicular phase stimulations:

Results of ovarian response to follicular phase stimulations with 1500-2000 I.U. of PMSG are shown in tables 1,3 and 4. Ovulations of three cows and one heifer treated with 1500 I.U. PMSG ranged from 1 to 6. The total number of the

ovulations were 10 with a mean of 2.50 ovulations. Ovulations of two cows and one heifer with 2000 I.U. PMSG ranged from 4 to 9, with a total of 19 and a mean of 6.33. Thus, a total of 29 ovulations were recorded with a mean of 4.14. The interval between gonadotrophin treatment and oestrus ranged from 3 to 4 days.

Luteal phase stimulations:

Results of the ovarian response to stimulations during luteal phase with 1500-2000 I.U. PMSG along with cloprostenol are given in tables 2,3 and 4. Ovulations from three cows and two heifers ranged from 4 to 7. The total ovulations were 29 with a mean of 5.80. Ovulations of two cows and one heifer subjected to 2000 I.U. PMSG injection ranged from 16 to 20, with a total of 53 and mean of 17.67. Thus, a total of 72 ovulations were recorded in five cows and three heifers treated with 1500-2000 I.U. PMSG with a mean of 9.00. The interval between the gonadotrophin treatment and oestrus was 5 to 6 days.

Ovarian response to follicular and luteal phase stimulations:

Table 3 gives the ovarian response to PMSG+cloprosterol during luteal phase and PMSG alone during follicular phase. Greater number of follicles ovulated in animals treated with 1500-2000 I.U. PMSG during luteal phase than in animals treated with PMSG in follicular phase. The mean ovulation rate of 10.30 in 8 animals given PMSG during luteal phase agreed with the observations of Elsden *et al.* (1974). The ovulation rates of 6.33 and 17.67 noticed with 2000 I.U. PMSG

TABLE 3: Effect of oestrous cycle phase on ovarian response in cows and heifers treated with 1500-2000 I.U. PMSG.

Phase of Oestrous Cycle	No. of animals treated	PMSG dose (I.U.)	Response of the ovary					
			Follicles			Ovulations		
			Total	Un-ovulated	Per cent ovulated	Total	Mean	Range
Follicular Phase	7	1500 to 2000	65	36	44.6	29	4.1	1-9
Luteal Phase	8	1500 to 2000	114	32	71.9	82	10.3	4-20

TABLE 4: Effect of PMSG dose on the ovarian response in cows and heifers treated during follicular and luteal phases.

PMSG Dose (I.U.)	No. of animals treated	Response of the ovary					
		Follicles			ovulations		
		Total	Unvoulated	Per cent ovulated	Total	Mean	Range
1500	9	59	24	59.3	35	3.9	1-7
2000	6	113	41	63.7	72	12	4-20

in follicular and luteal phases respectively were also observed by Church and Shea (1977).

Two cows and one heifer showed no ovulation on the right ovary in follicular phase stimulation. Hafez *et al.* (1963) and Sugie *et al.* (1972) advocated the use of HCG to induce ovulations in cows superovulated during the follicular phase. However, Dowling (1949), Hafez *et al.* (1965) and Laster (1973) opined that exogenous LH injection was not necessary since the bovine pituitary has high LH content. The PMSG injected on day 16 of the oestrous cycle might have stimulated the development of all follicles in the follicular wave. Thus follicles with viable oocytes ovulated and those with degenerated cocytes did not ovulate (Goodman *et al.*, 1977).

Luteal phase stimulations on day 10 of the oesrous cycle with PMSG and cloprostenol produced a reliable superovulatory response. This was attributed to the absence of a substantial follicular atresia by Rajakoski (1960).

Brock and Rowson (1952), Scanlon *et al.* (1968) and Henricks *et al.* (1973) recorded higher ovarian responses when the interval between PMSG injection and subsequent oestrus was 4 to 5 days. The interval was 3-4 days during follicular phase and 5-6 days in luteal phase stimulations. Thus, a better response was obtained in luteal phase stimulations.

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Studies On Gestational Oestrus In Surti Buffaloes

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ABSTRACT

Incidence, stage, signs, frequency and intensity and the effect of parity on occurrence of gestational oestrus were studied in Surti buffaloes based on the data of 7043 oestruses and 821 pregnancies recorded among 3875 animals, brought at the Veterinary College A.I. Clinic, Anand. There were 45 (0.64%) gestational oestruses among 42 (5.12%) pregnant animals with a mean gestation period of 82.42±5.47 days. In all 84.45% of the buffaloes showed gestational oestrus upto 120 days from fertile service/A.I. and the rest (15.55%) of them showed within 121 to 200 days.

The predominant signs of gestational oestrus in comparison to cyclic oestrus were: congestion of vaginal mucus membrane 64.44% vs 86.81%, the difference being significant ($P<0.05$); frequent micturation 48.89% vs 83.68%, withholding of milk 40.00% vs 75.93% and clean mucus discharge 24.44% vs 83.66%, the differences being highly significant ($P<0.01$). While bellowing was observed in 57.78% vs 68.76% and restlessness and excitement in 46.47% vs 56.17%, the differences being non-significant. Mounting behaviour was observed in only 3 (6.67%) pregnant buffaloes. Cervical mucus fern pattern during gestational oestrus was mostly atypical to nil in type. All, except two, animals exhibited single gestational oestrus.

Incidence was three times higher in buffaloes (6.05%) than the heifers (2.07%). The proportion of animals exhibiting gestational oestrus was highest (28.75%) in 3rd lactation buffaloes followed by 2nd (23.81%), 1st and 4th (14.29% each) lactation. The occurrence was found to be low in heifers (9.52%) and in animals above 5th lactation. Among gestational oestrus cases, pregnancy was 44.80% in left & 55.20% in right horn, the difference being non-significant.

* * *

Oestrus is usually not exhibited/noticed during gestation period in mammals. But this phenomenon seems to have a field practical importance with the productive and reproductive performance/efficiency of farm animals. There is a likelihood that in absence of accurate clinico-gynaecological examination for pregnancy, such animals may be sold as infertile or else abortion may occur if inseminated or served.

The incidence of gestational oestrus has been reported in Indian and exotic breeds of cattle and their crosses (Hall *et al.*, 1958; Erb and Morrison, 1958; Luktuke *et al.*, 1964; Chaudhary *et al.*, 1964; Sharma *et al.*, 1968; Sinha, 1972; Srivastava, 1978; Singal *et al.*, 1978; Patil *et al.*, 1982), in buffaloes (Johari, 1960; Chauhan *et al.*, 1976) and in sheep

(Lamond, 1963; Kandasamy and Pant, 1980). However, the detailed study on gestational oestrus in buffaloes is not fully documented. Because of paucity of such information in Surti buffaloes, the present study was conducted.

Materials and Methods

Data were collected on 7043 oestruses and 821 gestations among 3875 Surti buffaloes, including matured heifer to 6th lactation buffaloes. These animals were brought at the Veterinary college A.I. Clinic, G.A.U., Anand by the farmers for either A.I. and subsequent follow-up or for pregnancy diagnosis following AI/NS elsewhere. The period of study was from 1st April, 1983 to 31st March, 1985 (2 years).

The occurrence of oestrus was detected in these animals by the individual owners and were presented to the clinic. The heat period was confirmed by observations, vaginal inspections and rectal palpation and the findings were correlated with the owners observations. Pregnancy diagnosis was carried out by rectal examination 45-60 days after the A.I./NS and suspected cases were rechecked and confirmed again after 15-20 days of first check. The records and/or correct history from the owner for AI/NS of the animals exhibiting signs of oestrus were obtained and checked before insemination, especially in cases of doubt or very early pregnancy and were confirmed by ovarian activity through rectal palpation. The animals showing oestrus during pregnancy were clinically investigated and the incidence, stage, signs, effect of parity, frequency and intensity of gestational oestrus were studied on the basis of records and service history. Cervicovaginal mucus was collected from some animals, wherever possible, to study the crystalization pattern. The data were analysed according to the methods of Snedecor and Cochran (1967).

Results and Discussion

The incidence of gestational oestrus and the period of occurrence are presented in the table 1 and 2, respectively. The results revealed that there were 45 (0.64%) gestational oestruses among 42 (5.12%) pregnant buffaloes. These findings are coinciding with the findings of Johari (1960) in water buffaloes but are lower than those reported by Luktuke *et al.* (1964), Sharma *et al.* (1968) and Sinha (1972) in cattle. Luktuke and Purbey (1985) observed the incidence of gestational oestrus in heifers, cows and buffalo cows to be 3.23%, 6.23% and 5.98%, respectively. Chauhan *et al.* (1976) reported the incidence of gestational oestrus as 14.4% in buffaloes and 20.3% in cows. However, the present

TABLE 1. Incidence of gestational oestrus in Surti buffaloes

1	2	3	4	5	6
Total No. of buffaloes studied	No. of oestrus periods observed	No. of gestational oestrus periods	No. of buffaloes pregnant	No. of buffaloes showing gestational oestrus	Mean time of gestational oestrus (days)
3875	7043	45 (0.64%)*	821	42 (5.12%)**	Mean ± SE 82.42 ± 5.47

* Percentage of total oestrus periods.

** Percentage of total pregnant buffaloes.

TABLE 2. Period of occurrence of gestational oestrus in Surti buffaloes.

Days of Pregnancy	Cases of gestational oestrus		Average pregnancy period from fertile oestrus/A.I.
	No.	Per cent	Mean ± S.E. (days)
00 — 40	8	17.78	33.75 ± 1.65
41 — 80	17	37.78	66.24 ± 2.52
81 — 120	13	28.89	102.69 ± 4.11
121—160	6	13.33	133.33 ± 2.79
161—200	1	2.22	178.00
Total	45	100.00	82.42 ± 5.47

findings in Surti buffaloes are nearer to the findings of Singal *et al.* (1978) and Patil *et al.* (1982) who reported 1.88% and 1.32% incidence in crossbred heifers and cows and 2.04% in Haryana herds respectively. The low incidence in the present study on field data may be attributed to poor vigilence of animal owners towards detection of oestrus during very early gestation and once the animal is declared pregnant and hence non-availability of such animals for further follow-up, which is possible under farm condition right from conception to full term gestation.

The period of occurrence of gestational oestrus varied from 28 to 178 days with a mean of 82.42±5.47 days of gestation. In all 84.45% animals exhibited gestational oestrus upto 120 days from fertile service/AI. While rest (15.55%) of them showed within 121 to 200 days. These results are in agreement with those of Hall *et al.* (1958), Luktuke *et al.* (1964) and Singal *et al.* (1978) in cattle. Excess level of oestrogen vis-a-vis the progesterone level, in the early period of gestation, might have been responsible for exhibiting oestrus without affecting the pregnancy (Cole and Cupps, 1969; Tomar, 1970). The comparatively higher but varying oestrogenic levels during gestation may bring about difference in early and late gestational oestrus. The serum binding proteins have been presumed to regulate the levels of oestrogen and progesterone during various stages of gestation, thus arresting the oestrus during pregnancy especially in later stage by counteracting the highest level of placental oestrogen with progesterone (Nalbandov, 1958). However, application of various sensitive methods like RIA to estimate various steroids are still needed to confirm this approach.

The predominent signs of gestational oestrus in comparison to cyclic oestrus have been shown in table 3. It can be seen that the frequency of oestrus signs exhibited by these animals during gestational oestrus were significantly different from the signs of cylic oestrus, being higher in later. Tumifaction and oedema of vulva was observed in 26.67% cases of gestational oestrus, while mounting behaviour was observed in only 3 (6.67%) pregnant animals of which 2 were heifers and one of 3rd lactation buffalo. Clean cervico-vaginal mucus discharge voided out among 11 (24.44%) pregnant buffaloes was examined after drying on clean glass slides which revealed crystalization pattern of mostly atypical to nil in type. These findings are in general agreement with observations of Singal *et al.* (1978) and Patil *et al.* (1982) in cattle.

TABLE 3. Predominent signs of gestational oestrus in Surti buffaloes.

Oestrus signs	Gestational oestrus (45)		Cyclic oestrus (349)$		Cal X^2 Values
	No. Observed	Per cent	No. Observed	Per cent	
Bellowing	26	57.78	239	68.78	1.76^{NS}
Uneasiness & excitement	21	46.47	196	56.17	1.68^{NS}
Frequent micturation	22	48.89	293	83.68	14.53**
Withholding of milk	18	40.00	265	75.93	17.32**
Congestion of vaginal mucus membrane	29	64.44	303	86.81	5.71*
Clean mucus discharge	11	24.44	292	83.66	35.66**
Tumifaction and oedema of vulva	12	26.67	—	—	—
Mounting behaviour	3	6.67	—	—	—

NS = Non-Significant; * $P < 0.05$; ** $P < 0.01$. $ Rao and Kodagali (1983)

As regards the effect of parity on occurrence of gestational oestrus (refer, Table-4), the incidence was three times higher in buffaloes (6.05%) than the heifers (2.07%) with a range of 4.84% to 8.68% in 1st to 6th lactation group buffaloes. The higher incidence in 6th lactation buffaloes in the present study may be due to less number of pregnant animals in this group. These findings are little lower than that of Luktuke *et al.* (1964) who reported the incidence of gestational oestrus as 4.80% in heifers and 7.61% in zebu cows.

The proportion of animals exhibiting gestational oestrus was highest (28.57%) in 3rd lactation buffaloes, followed by 2nd (23.81%), 1st & 4th (14.29% each) lactation groups. The occurrence was found to be low in heifers (9.52%) and in animals above 5th lactation. Among gestational oestrus cases, pregnancy/ gravidity was 19 (44.80%) in the left horn and 23 (55.20%) in the right horn, the difference being statistically non-significant.

Further observation on intensity and recurrence showed that all animals have expressed a single normal oestrus except that one buffalo (4th lactation) showed pronounced gestational oestrus twice and one heifer thrice by about 12 to 15 days apart, in the same gestation. This is in agreement with the reports of Luktuke *et al.* (1964) and Patil *et al.* (1982). It is interesting to note that the above two exceptional cases in gestational oestrus behaviour were served by buffalo bulls which resulted in abortion within a week of service. The cause of abortion due to

TABLE 4. Effect of parity on occurrence of gestational oestrus.

Lactation Order	0(H)	1st	2nd	3rd	4th	5th	6th	overall
No. of Buff. Showing gestational oestrus	4	6	10	12	6	2	2	42
Percentage	9.52	14.29	23.81	28.57	14.29	4.76	4.76	100%
No. Pregnant	193	124	157	190	93	41	23	821
Per cent gestational oestrus	2.07	4.84	6.37	6.32	6.45	4.88	8.68	5.12

natural service by some bulls during gestational oestrus is not known but may be perhaps attributed to high level of seminal prostaglandin.

The causes for arrestment of oestrus and ovulation during pregnancy are still obscure which may involve intrinsic as well as extrinsic factors. Supression of oestrus in pregnancy seems to be due to progesterone secreted by newly formed corpus luteum as pregnancy ensues. Luktuke *et al.* (1964) have reported that in the initial stage of pregnancy, considerable follicular growth is observed in the ovaries, which might be the attributing cause for gestational oestrus due to increased level of oestrogen and its metabolites in blood. According to Williams *et al.* (1963) and Mirskaia and Smirnov (1941), heats during pregnancy are not accompanied by ovulation and generally no superfoetation takes place in bovines.

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Histochemical Observation On Endometrium Of Cows In Relation To Fertility: Glycogen And Alkaline Phosphatase Activity

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ABSTRACT

Studies on glycogen and alkaline phosphatase activity revealed marked difference between fertile and repeat breeder cows. It was observed that in the group of animals where the score value for glycogen was more than 0.5, 63.63 percent conceived on first A.I. post-biopsy as compared to the percentage of 26.53, where the score value was less than 0.5. It was further observed that a low activity of alkaline phosphatase (score value less than 0.5) was more conducive for fertility. The possible role of the two components in the process of conception has been discussed.

* * *

Nutritive material primarily glycogen is gradually stored in the uterine epithelium and musculature under the influence of estrogen (Olds and Van Demark, 1957) to be metabolised and utilized later by the implanting blastocyst and also to maintain the dynamic state of uterus (Hughes *et al.*, 1963). Maximum alkaline phosphatase activity in the endometrium has been reported to occur during progestational phase (Marinov and Lovell, 1968) and besides performing other functions has been linked with transfer of solutes across the membrane of secretary cells (Connel, 1972). The present paper records the localisation of glycogen and alkaline phosphatase activity during fertile and infertile estrus in cows.

Materials and Methods

The study was conducted on 71 cows of the University farm, Hissar. The animals were maintained under optium managerial conditions. All the animals included in this study had apparently normal genitalia and did not reveal any palpable genital abnormality on rectal examination. They were checked for the absence of brucellosis and Johne's disease. Heat detection was performed regularly every morning and evening with help of vasectomised bulls and trained attendants. Prior to biopsy the cows were grouped as follows:

Group I. Animals which were either fresh calvers or had up to two artificial inseminations (A.I.) before endometrial biopsy were considered normal.

Group II. Animals which had been inseminated three or more times but had not conceived and were, therefore, classed as repeat breeders.

Biopsy specimens were obtained with the help of a modified Neilsen's biopsy catheter as per the technique adopted by Sinha *et al.* (1983). The biopsy samples were preserved in chilled, neutral buffered 10% formalin and were processed through the standard acetone—benzene schedule (Lillie, 1947). Paraffin sections, 4 to 6 microns thick, were stained using standard procedures for glycogen (Periodic Acid Schiff reaction; McManus 1946 with and without diastase and Best's carmine; Humason 1967) and alkaline phosphatase (Gomori, 1946).

Quantitation of histochemical reaction:

The following scale was adopted for quantitative evaluation of the cytochemical results in individual components of endometrium. Light — Fine deposits of histochemical material along the cell outline or in the cytoplasm—grade I. Moderate—continuous reaction with occasional fine or coarse granules or blebs — grade II. Intense — Patchy deposition of histochemical material obscuring the intercellular outline and masking the individual of the cells—grade III. Very intense—Histochemically reactive material altogether obscuring the cellular and nuclear identity — grade IV.

Total score of the individual endometrial tissue factor:

This factor was obtained by multiplying 4 (grades) with the number of endometrial components. The animals were graded into three grades of fertility on the basis of pregnancy results as described earlier (Sinha *et al.*, 1983).

Results

The concentration of PAS positive, diastase labile and Best's carmine positive

Fig. 1. Microphotograph showing concentration of PAS positive granules in the supra nuclear part of the luminal epithelial cells with a clear infranuclear area. Basement membrane is intensely PAS positive. McManus PAS × 400.

material (glycogen) was found to be maximum in the luminal epithelium and basement membrane (Fig. 1). The staining intensity in the superficial stroma was nearly double as compared to the deeper stroma and was comparable to deeper glands. Occasionally large PAS positive granules were observed in the superficial glandular epithelial cells and the luminal contents were intensely PAS reactive (Fig. 2). Interpretation of quantitative data on the localization of glycogen in different components of endometrium revealed that deposition was always higher in fertile group (grade I)

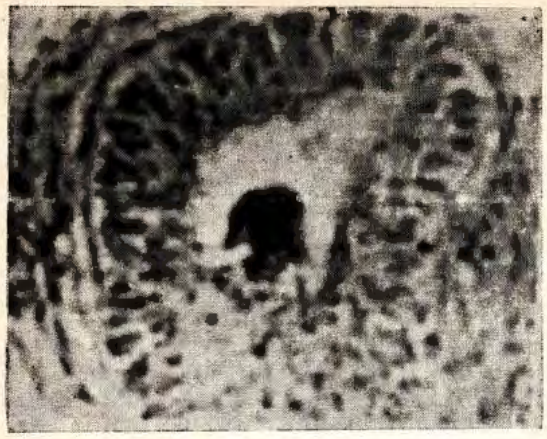

Fig. 2. Microphotograph showing large PAS positive granules in glandular epithelial cells, the luminal content is intensely PAS reactive. McManus PAS × 400.

as compared to subfertile (grade II & III). It was true for all the five components of endometrium. It was further observed that as the score value of glycogen decreased below 0.5 there was an increase in the percentage of grade II and III fertility types of animals (73.47%), which did not conceive on first A.I. after biopsy. Reverse was found to be true for the group of animals showing a glycogen score above 0.5, wherein 63.63 percent of animals conceived on 1st A.I. post biopsy (grade I fertility) and only 36.36% of the animals were of grade II & III types (Fig 3). Chi-square test revealed that there was a significant difference between grade I and II ($P<0.01$) and grade I and III ($P<0.05$), but grade II animals did not differ from grade III type (Tables, 1,2,3).

The activity of alkaline phosphatase was observed to be present in supranuclear portion of the luminal and glandular epithelium (Fig 4). The nuclei

TABLE 1: Comparison of two grades (I and II) of cows in relation to glycogen distribution score

	Grade I		Grade II		Total	Chi-square
Score	Observed	Expected	Observed	Expected		value
Less than 0.5	13	17.18	15	10.82	28	7.244**
More than 0.5	14	9.82	2	6.18	16	

** Significant at 1 per cent level

TABLE 2. Comparison of two grades (I and III) of cows in relation to glycogen distribution score

	Grade I		Grade III			Chi-square
Score	Observed	Expected	Observed	Expected	Total	value
Less than 0.5	13	17	21	17	34	
More than 0.5	14	10	6	10	20	5.08*

* Significant at 5 per cent level

TABLE 3. Comparison of two grades (II and III) of cows in relation to glycogen distribution score

	Grade II		Grade III			Chi-square
Score	Observed	Expected	Observed	Expected	Total	value
Less than 0.5	15	13.91	21	22.09	36	
						0.767NS
More than 0.5	2	3.09	6	4.91	8	

NS: Non-Significant difference

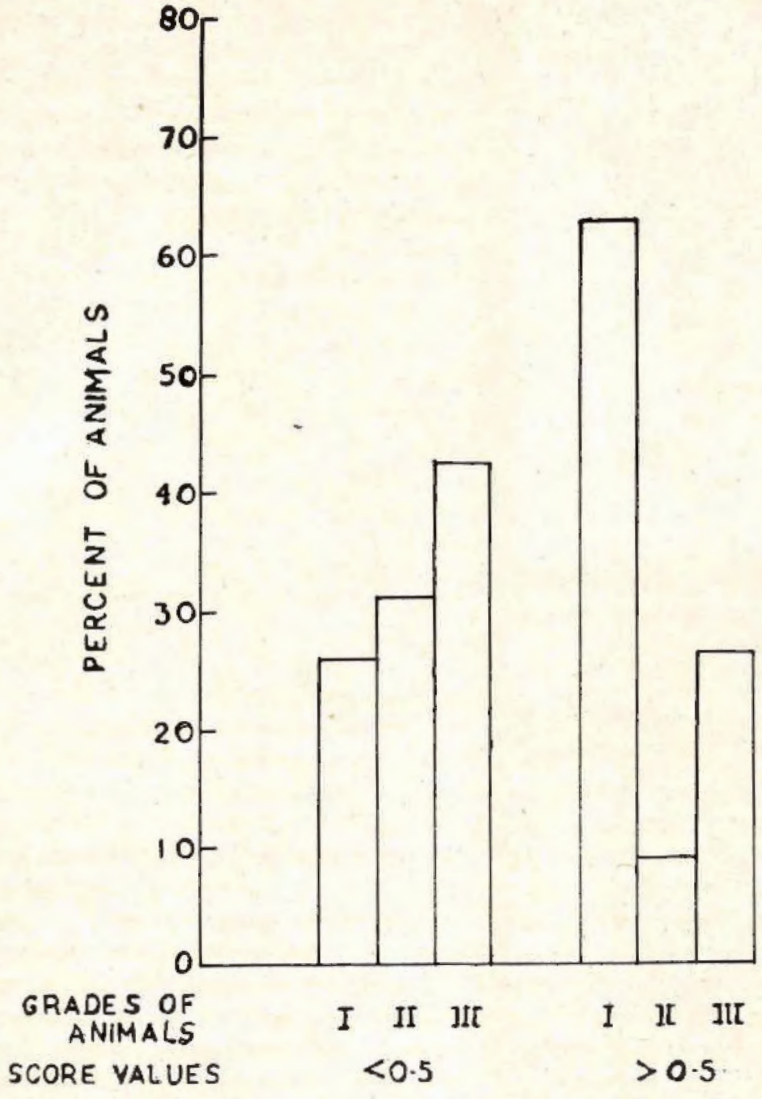

FIG. 3 GLYCOGEN DISTRIBUTION SCORE IN ENDOMETRIUM OF COWS IN DIFFERENT GRADES OF FERTILITY

Fig. 4. Microphotograph showing moderate reaction for alkaline phosphatase in the apical portion of glandular epithelium. The luminal contents are very strongly reactive. Gomori alkaline phosphatase reaction × 400.

of the glandular contents wherever present showed an intense to very intense degree of reaction. The luminal epithelium and superficial glands showed a reaction of almost very intense degree. The reaction in superficial stroma and deeper glandular epithelium was almost comparable. The deeper stroma was least reactive. It was observed that in the group of animals showing a reaction below 0.5 the percentage of grade I animals was higher (42.86%). In the other group showing a reaction above 0.5, the percentage of grade I animals decreased to 33.33 percent (Fig 5). It appeared that a lower intensity of reaction was conducive for good fertility.

Discussion

Maximum concentration of glycogen granules was found to be in the luminal epithelium followed by superficial and deep glands in descending order. This is in agreement with the previous reports of Moss *et al.* (1954), Marinov and Lovell (1968) and Larson *et al.* (1970). Olds and Van Demark (1957) reported that estrogens stimulated the deposition of glycogen in the uterine epithelium and musculature. Its movement from the basal ends to the lumen and release was influenced by progesterone. During the present investigation it was observed that overall glycogen concentration in the endometrium bore a direct relationship with the level of fertility in cows. As the score value increased from 0.5 and above, the percentage of animals with grade I fertility increased and when the score value dropped to less than 0.5 there was a sharp rise in the percentage of subfertile animals (grade II & III). In sterile women diminition of glycogen in the endometrium has been observed by Hughes *et al.* (1963). The glycogen accumulated during the estrogenic phase is released during progestational phase to serve as an energy source for the free floating blastocyst. Therefore, less accumulation of glycogen during estrogenic phase may result into less availability to the blastocyst which may lead to its death due to starvation and the animal repeats. In addition to supply nutrition to the fertilized ovum, glycogen is further required to maintain high energy metabolism of endometrium (Larson *et al.*, 1970). An improperly developed endometrium may be either weak or not readily suitable to bear the developing embryo leading to its death and repeatition of a new estrus cycle. Most of these early embryonic deaths are reported to take place before day 16 (Boyd *et al.*,

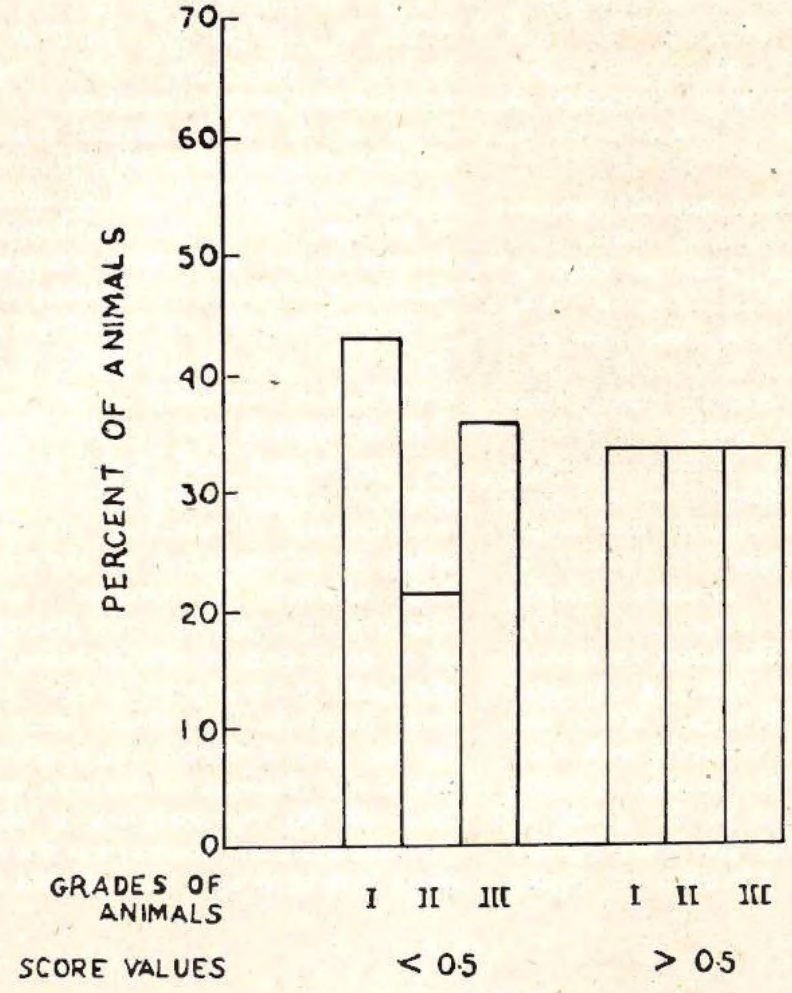

FIG 5. ALKALINE PHOSPHATASE DISTRIBUTION SCORE IN ENDOMETRIUM OF COWS IN DIFFERENT GRADES OF FERTILITY

1969) in which oestrus cycle lengths are not affected (Roberts, 1971). Wordinger *et al.* (1971) observed that in melenogesterol treated heifer's endometrium, accumulation of glycogen occurred less as compared to control and also rate of fertilization and number of developing young embryos reduced. It was interpreted that in such animals either there was a diminished secretion of estrogen or a failure of endometrium to respond to estrogen.

The results revealed that the general reaction of endometrial tissue for alkaline phosphatase was moderate to high. The maximum reaction was observed in the luminal epithelium and the superficial glands followed by superficial stroma and deeper glands. This pattern of reactivity gradient is in conformity with earlier findings of Moss *et al.* (1954) and Marinov and Lovell (1968). It has been reported that maximum alkaline phosphatase activity in the endometrium occurred during progestational phase. Larson *et al.* (1970) and Moss *et al.* (1954) found an inverse relationship between levels of glycogen and alkaline phosphatase. When the fertility results were correlated with score value of alkaline phosphatase reaction it was observed that in the group of cattle where the score was below 0.5 the percentage of grade I fertility was higher as compared to that of grade II and III fertility. Whereas, in the group of cattle with score value above 0.5 the percentage distribution of animals in all three fertility groups was alike. The major functions of alkaline phosphatase are to help the transfer of solutes across the cell membrane, to catalyse the hydrolysis of a variety of phosphate esters and in carbohydrate metabolism (Connel, 1972). Moss *et al.* (1954) stated that the alkaline phosphatase activity is necessary for glycogen utilization and accumulation of glycogen occurs because phosphatase activity is low or absent. Nonetheless, alkaline phosphatase and glycogen may be present together but where this situation exists, the concentration of phosphatase is relatively low.

In the light of present results it could be hypothesised that during estrogenic phase of a normal fertile cycle the low concentration of alkaline phosphatase helps in accumulation of glycogen. A high alkaline phosphatase activity observed in subfertile cows during this phase might indicate that mobilization of glycogen has already set in, much earlier than required. The result being that by the time fertilized ova reaches the uterus and develops to blastocyst, energy stock in the endometrium is already exhausted and the blastocyst is starved to death leading to repeatition of estrus cycle.

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Levels Of Serum Enzymes In Primary Infertile and Normal Cyclic Kankrej Heifers

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ABSTRACT

The concentration of various enzymes and interrelationship among them iu serum of normal cyclic and primary infertile heifers was studied. The mean values for AKP, ACP, GOT, GPT and LDH were 4.98±0.75 and 7.37±0.45, 0.36±0.15 and 0.087±0.01 (BLunits/ml.) 30.34±5.38 and 32.36±5.35, 65.29± 13.98 and 30.23±4.70 (µmole/min/lit); 885.36±6.35 and 874.47±8.54 (iu/lit) in normal and primary infertile groups respectively. The ACP content was significantly & positively correlated with AKP and GPT contents in serum of normal cyclic heifers. The LDH was also found to be significantly interrelated with GOT content in same group. Whereas, none of the estimated characters was significantly correlated in infertile group of heifers. Both phosphatases and GPT could be considered as an indicator of diagnostic tools of primary infertility. The results are required to be confirmed on large number of animals.

* * *

It is fact that the sound and economic animal production is dependable on reproductive efficiency of an animal. The reduced reproductive efficiency could be attributed to a number of causes of which primary infertility is the major drawback to the low reproductive efficiency. The large number of factors viz. Endocrine disturbances, poor nutrition, seasonal influences and systemic diseases might be attributed to the cause of primary infertility. The study of serum enzymes has not been reported in animals maintained under uniform managemental conditions. Therefore, an attempt has been made on Kankrej heifers having problem of primary infertility, to investigate the levels of various serum enzymes, so as to ascertain their possible involvement and usefullness as tools of infertility diagnosis.

Materials and Methods

The investigation was conducted between two groups of heifers viz. Normal cyclic and primary infertile. The heifers, who did not manifest any sign of cestrus even after attainment of pubertal age, were considered as primary infertile. The primary infertile group consisted of fourteen heifers while another group of normally cycling heifers consisted of ten animals. The animals under study were examined per-rectally for absence of any anatomical deformities of genitalia. The heifers were kept under uniform managemental conditions at Livestock Research Station, Gujarat Agricultural University, Sardar Krushinagar. The serum was separated from the blood samples collected from the jugular vein.

TABLE 1: Levels of various enzymes in serum of normal cyclic and primary infertile heifers.

Sr. No.	Serum Characteristics	Normal heifers (N = 10) Mean ± S.E.	Primary infertile heifers (N = 14) Mean ± S.E.	Calculated 't' Value
1.	Alkaline Phosphatase — AKP (B.L. Units/ml)	4.98 ± 0.75 (2.77 — 9.86)	7.37 ± 0.45 (3.56 — 9.00)	2.89**
2.	Acid Phosphatase — ACP (B.L. Units/ml)	0.36 ± 0.15 (0.08 — 1.28)	0.087 ± 0.01 (0.03 — 0.24)	2.10*
3.	AKP/ACP ratio	24.41	127.73	—
4.	Glutamic Oxaloacetic Transaminase — GOT (µmole/min/lit)	30.34 ± 5.38 (11.82 — 70.92)	32.36 ± 5.35 (1.97 — 66.98)	0.26
5.	Glutamic Pyruvic Transaminase — GPT µmole/min/lit)	65.29 ± 13.98 (12.02 — 169.26)	30.23 ± 4.70 (12.09 — 60.45)	2.91**
6.	GPT/GOT ratio	2.29	3.49	—
7.	Lactic Dehydrogenase — LDH (iu/lit)	885.36 ± 6.35 (853.65 — 926.82)	874.47 ± 8.54 (828.05 — 951.25)	0.95

The figures in parenthesis indicate range values. **Significant at 1% * Significant at 5%
N = No. of observations

The serum vials were stored at —20°C until analysed for biochemical constituents. The standard procedures were followed for alkaline and acid phosphatases (Sigma Technical Bulletin no. 104); Glutamic oxaloacetic transaminase and pyruvic transaminase (Oser, 1974) and lactic dehydrogenase (Wootton and Freeman, 1982) respectively.

The obtained values of aforementioned enzymes were subjected to student 't' test (Snedecor and Cochran, 1972). The interrelationship of estimated enzymes was studied by correlation coefficients.

Results and Discussion

The mean values±S.E. of AKP, ACP, GOT, GPT and LDH in serum of normal cyclic and primary infertile heifers are presented in table 1. The summary of interrelationship among the various enzymes in both the group of heifers has been given in table 2.

The values of AKP of normal cyclic and primary infertile heifers were 4.98 ±0.75 and 7.37±0.45 BL Units/ml respectively. The differences between these values were highly significant ($P<0.01$) which is in agreement with the

TABLE 2: Correlation coefficients among various serum enzymes in normal cyclic and primary infertile heifers.

Sr. No.	Characteristics	Normal Cyclic Heifers				Primary Infertile Heifers			
		ACP	SGOT	SGPT	LDH	ACP	SGOT	SGPT	LDH
1.	AKP	0.642*	—0.384	—0.029	—0.074	0.208	0.285	—0.269	0.269
2.	ACP		—0.317	0.508*	—0.376		—0.099	—0.059	—0.033
3.	SGOT			0.039	0.718*			—0.296	—0.537
4.	SGPT				—0.307				0.398

* Significant at 5%.

findings of Derashri *et al.* (1984). Devraj (1983) reported that AKP enzymes showed a tendency being low in concentration towards the follicular development. So, it may be said that decreased concentration of AKP in normal cyclic heifers might enhance the folliculogenesis and further may increase the pace of conception, while reverse is true in primary infertile heifers. The AKP was also found to be significantly & positively correlated with ACP in normal cyclic heifers.

The mean values of ACP were significantly higher in normal cyclic heifers which are in agreement with the findings reported by Roussel and Stallcup (1967). King *et al.* (1945) and King (1971) reported that ACP level is an useful index for assessment of the estrogen level in heifers. The findings of present study seem to be conceivable with above theme. So much so, increased concentration of ACP might be helpful in hydrolysing the organic phosphomonoesters and thus may provide energy in the form of phosphates in normal cyclic animals. The ACP contents were also found to be significantly & positively correlated with GPT contents in normal heifers. The trend for both phophatases value is opposite to each other in both groups of heifers. The AKP/ACP ratio was 24.41 and 127.73 in normal & primary infertile group of heifers, respectively.

The level of SGPT was slightly higher in primary infertile heifers but differences between two groups of heifers were not significant which is in agreement with the findings of Davis *et al.* (1965) and Derashri (1982). They have reported the SGOT activity in anoestrus state of reproduction. It was found to be significantly positively interrelated with LDH in normal heifers. Further, Roussel and Stallcup (1967) reported that change in SGOT activity is due to hormonal changes in body rather than body stress. The range of enzymatic activity was narrow in the infertile heifers.

The level of SGPT was significantly lower in the infertile group of heifers as compared to normal group. The findings of present investigation are in agreement with the observation of Derashri (1982). He reported the same trend of SGPT concentration in anoestrus condition. Both these findings suggest the possible involvement of hormonal levels prevailing during normal oestrus cycle, in control of SGPT levels. However, such a direct evidence is not available in the support of above theme.

Henry *et al.* (1974) profound that LDH helps in the conversion of lactic acid into pyruvic acid. The increase trend of LDH in normal cyclic heifers might be responsible for production of more pyruvic acid which in turn provides energy in better way for the normal reproductive functions than those of infertile heifers. However, the difference in mean values of LDH was not significant in both groups. The LDH correlated significantly in a positive way with GOT contents of normal group whereas it was found to be negatively correlated with rest of enzymes in same group of heifers.

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Plasma FSH And LH Concentrations (After Cloprostenol Injection) At Pro-oestrus And Oestrus In The Dairy Cows

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ABSTRACT

Plasma FSH and LH concentration was monitored in dairy cows after giving cloprostenol 100 μg i.m. on Day 14 of the oestrous cycle. Two hours after the cloprostenol injection, there was an immediate increase in plasma LH concentration in 1 of 5 cows (Cow X-430) and an increase in plasma FSH concentration in 1 of 5 cows (Cow P-126) at 4 h. The FSH concentration varied to a greater exent from one cow to another.

Four of 5 cows showed the pre-ovulatory FSH surge and in 3 cows, the LH surge. The time of occurrence of these peaks varied between cows. The mean time leg between the cloprostenol injection and gonadotrophin surges was as follows: FSH 35.5±10.9 h and LH 36.67±13.9 h. The peak concentration of FSH was 6.09 ±1.94 ng/ml and LH 23.67±11.23 ng/ml.

* * *

In the cow, the hormonal changes after the onset of cloprostenol-induced or natural oestrus have been reported by various workers (Akbar *et al.*, 1974; Dobson *et al.*, 1975; Schams *et al.*, 1977; Dobson, 1978; Butler *et al.*, 1983). We measured the concentration of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in the jugular plasma of cows before and after the administration of cloprostenol, a prostaglandin $F_2\alpha$ analogue.

Materials and Methods

Cloprostenol, (Estrumate ICI), an analogue of prostaglandin $F_2\alpha$ was injected i.m. in two doses of 100 μg each, 12 days apart, to 5 upgraded (Zebu × Holstein) cows. Subsequent to this synchronisation of oestrus, i.e. on Day 11 of the oestrnus cycle normal saline (0.85% w/v NaCl) 1 ml was injected i.m. On Day 14, 100 μg of cloprostenol was injected i.m. Every 4 hours, the cows were examined and observed for clinical signs of oestrus, and every day the genitalia was palpated per-rectally.

Radioimmunoassay of gonadotrophins:

Using the ovine gonadotrophin label (oFSH,G4-211B donated by Professor Papkoff to Professor Moudgal and oLH, LER-1374A-NIAMDD) and standards, the concentrations of FSH and LH were measured by a double antibody radio-immunoassay method. The specificity of antibodies against FSH and LH used in the present study was reported by Rao *et al.* (1974). Assaying a plasma sample of a cow having low hormone concentration, the respective inter and intra-assay coefficients of variation were 14.3% (n=4) and 12.2% (n=4) for FSH and

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18.1 and 13.0% for LH. Assaying a plasma sample of high hormone concentration, the respective inter and intra-assay coefficients of variation were 18.0 and 14.4% for FSH, and 14.5 and 12.9% for LH. The results are expressed as ng equivalents/ml of LER-1818-2-oFSH for the FSH assay and 21-NIAMDD-oLH for the LH assay. The sensitivity of the method for either FSH or LH was 0.1 ng/tube.

Results and Discussion

PLASMA GONADOTROPHIN CONCENTRATIONS IN THE CONTROL PERIOD (before cloprosteuol injection—saline on Day 11 of the oestrous cycle):

In the control period, occasionally, there were small synchronous increases in plasma FSH and LH concentrations. Small increments of plasma FSH concentration occurred at intervals of 6 to 40 h (19.5$\pm$6.7 h). The concentration of gonadotrophins, especially FSH varied to a greater extent from one cow to another. For example, in cow X-420, small increase in FSH concentration was discernible at intervals of 24 to 30 h (17.33$\pm$6.07 h) and small increase in LH at intervals of 4 to 20 (26.5$\pm$6.8) h. These small increases of FSH and LH did not coincide with each other in this cow. In other control cows, small increases in FSH and LH were not clearly evident.

The basal plasma FSH concentration was 5.97$\pm$0.25 ng/ml (n=77) and LH, 0.61$\pm$0.15 ng/ml (n=71). Because of high LH concentration in a cow X-420, LH peak was evident on two occasions before the preovulatory LH surge.

PLASMA GONADOTROPHIN CONCENTRATIONS AFTER THE CLOPROSTENOL INJECTION:

Immediate changes (i.e. at pro-oestrus)—Out of 5 cows, 1 cow (X-430) showed an immediate increase in plasma LH concentration (2 h after the cloprostenol injection; the first sample after injection) and one cow (P-126) showed a small increase in plasma FSH concentration 4 h after the cloprostenol injection.

Later changes (Ovulatory surges i.e. at oestrus): Out of 5 cows, 4 cows (P-84, P-85, P-126 and X-420) showed a preovulatory FSH surge and three cows (P-85, P-126 and X-420), the LH surge. To a greater extent, the time occurrence of these peaks varied from cow to cow. The mean time lag between the gonadotrophin surges and the cloprostenol injection was as follows: FSH 35.5$\pm$10.9 h (84 h for P-85, 12 h for P-126 38 h for X-420, 28 h for P-84) and LH 36.67$\pm$13.9 h (82 h for P-85, 14 h for P-126 and 34 h for X-420). The peak concentration of FSH was 6.09$\pm$1.94 ng/ml and LH 23.67$\pm$11.23 ng/ml.

The first FSH surge occurred 18.0$\pm$9.25 h (n=3) before the onset of behavioural oestrus except in one cow (P-85), in which it occurred 18 h after the onset of behavioural oestrus. Out of 3 cows which showed LH surge, in two cows (P-126 and X-420) LH surge was evident 17.0$\pm$15.04 h before the onset of the behavioural oestrus and in the other cow (P-85), LH surge was noted 16 h after the onset of the behavioural oestrus. Although, a peak LH surge was evident in cow X-430, a small increase in LH concentration was noted at 34 and 62 h after the cloprostenol injection. This cow showed a maximum FSH concentration at 68 h. An asynchronous FSH peak was seen at 38 h in the cow X-420.

Among the cows, where blood sampling was continued beyond 1 to 2 days after

LH peak, 2 cows (X-420 at 64 h and P-126 at 70 h after the cloprostenol injection) showed a second maximum rise in plasma FSH concentration unaccompanied by any increase in LH.

Three days after the oestrus, in all the cows, a single corpus luteum was palpated.

Soon after cloprostenol injection, progesterone concentration started to decline and reached the minimal concentration of 0.1 to 0.2 ng/ml on the day of oestrus. The progesterone profile subsequent to the experiment was characterised by maximum progesterone concentration on Day 10 to 12 of the oestrous cycle.

The present investigation revealed that during the luteal phase of the oestrous cycle, small increments (pulsatile secretion) of FSH and LH secretion occur in a regular manner. Schams *et al.* (1977) have reported distinct FSH peaks on Day 17-18 of the oestrous cycle. The analogous situation of clopostenol-induced oestrus and pro-oestrus of the present study confirms the finding of such increases of FSH as reported by Schams *et al.* (1977) and that of LH as reported by Dobson *et al.* (1975).

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Immunological Studies Of Bovine Semen In Relation To Fertility

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ABSTRACT

Semen samples from four bulls were taken to study the agglutination reaction with the cervical mucus of repeat breeding and normal breeding animals. It was observed that the agglutination reaction with the cervical mucus against the washed sperm antigen in repeat breeding heifers and cows was 38.89% and 29.42% respectively, where as against whole semen antigen, it was observed to be 11.11% and 11.76% respectively.

The agglutination reaction was more intense in cervical mucus than the serum when treated with washed sperm antigen than whole semen antigen, which was revealed by higher titre obtained in the present investigation. In the serum the antibody titres were lower than the cervical mucus.

It was observed that out of 37 reactors (71.15%), 10 (27.03%) conceived whereas from 15 non-reactors, 10 (66.67%) conceived.

* * *

The important criteria for an individual dairy man or a herd owner to obtain a satisfactory productivity level lies in the investigation of infertility and sterility conditions in cattle. These reproductive failure may be associated with various forms of infertility conditions either singly or combinedly. So the introduction of superior germ plasm for genetic improvement and higher economic production has created more importance for evaluation of male infertility. The precise mechanism by which the antisperm antibodies might influence in vivo with reproduction are unknown. However, proposed mechanism include: agglutination or immobilisation of spermatozoa, impairment or prevention of sperm penetration in the cervical mucus, interference with capacitation and embryo implantation and finally the enhancement of phagocytosis (London *et al.*, 1984). So the present investigation was undertaken to study the antigenic nature of bovine semen, to detect whether any antisperm antibodies produced locally in the genital tract or circulatory antibodies in the serum, to conduct immunological agglutination and finally to study the effect of local and circulatory antibodies on the fertility level of cows.

Materials and Methods

The present study was carried out in the Department of Gynaecology, Orissa College of Veterinary Science and Animal Husbandry, Bhubaneswar. A total of 44 cows and 24 heifers presented for artificial insemination during oestrus at Central Clinics were taken for the present investigations. These animals were divided into two groups:

1. Normal breeding group used as control animals consists of 6 heifers in

TABLE 1: Results of agglutination test of repeat breeding and normal breeding cows and heifers.

Animals		Washed sperm antigen				Whole semen antigen			
		Cervical mucus		Serum		Cervical mucus		Serum	
		+ Ve	— Ve	+ Ve	— Ve	+ Ve	— Ve	+ Ve	— Ve
Repeat breeding 52*	Heifers 18*	38.89% (7)	61.11% (11)	16.67% (3)	83.33% (15)	11.11% (2)	88.89% (16)	11.11% (2)	88.89% (16)
	Cows 34*	29.42% (10)	70.58% (24)	17.65% (6)	82.35% (28)	8.82% (3)	91.18% (31)	11.76% (4)	88.24% (30)
Normal breeding 16*	Heifers 6*	—	100.00% (6)	—	100.00% (6)	—	100.00% (6)	—	100.00% (6)
	Cows 10*	20.00% (2)	80.00% (8)	—	100.00% (10)	—	100.00% (10)	—	100.00% (10)

* Indicate number of animals under study.
Figures in parentheses indicate number of samples under study.

their post pubertal heat and 10 cows in their first post partum heat (within 60-90 days).

2. Repeat breeding group which constituted 34 cows and 18 heifers which had failed to conceive after repeated inseminations.

The breeding history of the animals were recorded and the animals having any sort of systemic infections on clinical examination were excluded from the present investigation.

Semen was collected from four bulls belonging to the semen collection centre, Bhubaneswar, stationed at the Department of Gynaecology, Orissa Veterinary College, twice a week. A total of 6 ejaculates from each bull were studied.

Three types of antigens were prepared for the present investigation: Whole semen antigen (Deo and Roy, 1971), seminal plasma antigen and washed sperm antigen. Antibody to be used in the investigation were prepared from cervical mucus and serum of the animals.

The spermatozoal agglutination test was conducted by taking antibody extract of cervical mucus and serum against the washed sperm antigen and whole semen antigen separately by the help of a plastic serological plate as described by Deo and Roy (1971). The intensity of agglutination was rated as described by Smith (1949).

The statistical analysis was done as per the method described by Snedecor and Cochran (1967).

Results and Discussion

The results of agglutination test with cervical mucus and sera from 52 repeat breeding and 16 normal breeding heifers and cows are presented in Table 1. The agglutination reaction with the cervical mucus and sera against the washed sperm antigen and whole semen antigen were not uniform for specific bulls used in the present investigation. More over in the repeat cows and heifers production of local antibodies in the genital tract or circulating antibodies in the serum might be a factor causing low fertility (Deo and Roy, 1971 and Bhatt *et al.*, 1979).

TABLE 2: Incidence of agglutination of spermatozoa at different titres of antibodies in the cervical mucus and serum of repeat breeding and normal breeding Cows and Heifers.

Animals		Washed sperm antigen						Whole semen antigen					
		Cervical mucus			Serum			Cervical mucus			Serum		
		L	M	H	L	M	H	L	M	H	L	M	H
Repeat breeding 52*	Heifers 18*	28.57% (2)	42.86% (3)	28.57% (2)	33.33% (1)	66.67% (2)	—	—	50.00% (1)	50.00% (1)	100.00% (2)	—	—
	Cows 34*	20.00% (2)	30.00% (3)	50.00% (5)	33.33% (2)	50.00% (3)	16.67% (1)	66.67% (2)	33.33% (1)	—	50.00% (2)	50.00% (2)	—
Normal breeding 16*	Heifers 6*	—	—	—	—	—	—	—	—	—	—	—	—
	Cows 10*	100.00% (2)	—	—	—	—	—	—	—	—	—	—	—

L = Low titre
M = Medium titre
H = High titre

*-Indicate number of animals under study.
— Figures in parentheses indicates number of samples under study.

Though positive reaction in both cervical mucus and serum against washed sperm and whole semen antigen was observed, still the variation in agglutination reactions in both the antigens were marked. The highest reaction being observed in cervical mucus (29.42%) in washed sperm antigen and lowest (8.82%) in whole semen antigen. The difference in the incidence of agglutination in serum with washed sperm (17.65%) and whole semen antigen (11.76%) revealed a difference as wide as compared to that of the cervical mucus antibodies. This difference in agglutination reaction between the whole semen and washed sperm antigen might be attributed to the immunological protective mechanism of seminal plasma in the whole semen as suggested by Tarter and Alexander (1984). They opined that seminal plasma contains agents that protects spermatozoa against immune assault both within the male genitalia or after ejaculation within the female, which might be the case in the present investigation for this variation.

In the present investigation the agglutination reaction with the cervical mucus against the washed sperm antigen & whole semen antigen in repeat breeding heifers & cows were 38.89% & 29.42% and 11.11% & 8.82% respectively. The incidence of agglutination in the serum against the washed sperm antigen & whole semen antigen in the repeat breeding heifers & cows were 16.67% & 17.65% and 11.11% & 11.76% respectively. This difference in result as compared with earlier investigators might be ascribed to the fact that the number of animals used in the present investigation is more & also to the individual susceptibility to immunological reaction.

The incidence of agglutination of spermatozoa at different titres of antibodies in the cervical mucus & sera of repeat breeding animals & normal breeding animals are presented in Table 2.

TABLE 3: Results of Immunological agglutination in relation to pregnancy.

Immunological test	Number of reactor animals				Pregnancy		Number of non-reactor animals	Pregnacy	
	Total	In W.Sp	In W.S	In S.P	No.	%		No.	%
Agglutination	37 (71.15)	26 (70.27)	11 (29.73)	—	10	27.03	15 (28.85)	10	66.67

W.SP. = Washed spermatozoa
W.S. = Whole semen
S.P. = Seminal plasma.

It was observed that a total of 7 positive cases showed agglutination reaction in the cervical mucus against the washed sperm antigen whereas only 2 cases were marked against whole semen antigen. In the present investigation the incidence of agglutination at high titre against whole semen antigen either with cervical mucus or with serum was lower than washed sperm antigen which might be due to immune protective mechanism of seminal plasma over spermatozoa.

The cervical mucus of 10 positive cases from among the 34 repeat breeding cows had agglutination at different titres. Three cervical mucus samples from repeat breeding cows were agglutinated in whole semen antigen at low and medium titre, whereas the agglutination pattern of the serum samples from the repeat breeding cows against the washed sperm antigen showed 33.33%, 50.00% and 16.67% at low, medium and high titres respectively; but no cervical mucus and serum samples from the cows had agglutination at high titre against the whole semen antigen.

Among the normal breeding animals positive agglutination reaction was also marked in 20% of cows at a very low titre of the cervical mucus against the washed sperm antigen. But no reaction was observed when tested against whole semen antigen. However, the cows which had agglutination did not have reproductive problems and conceived normally. It is thus postulated that the agglutination observed is of nonspecific in nature, which is in close agreement with the findings of Deo and Roy (1971). From the present investigation it is also evident that the incidence and intensity of agglutination reaction in the cervical mucus of repeat breeding animals was more intense against the washed sperm antigen than the whole semen of specific bulls, compared to normal breeding animals. As the cervical mucus showed higher agglutination reaction than the serum in both the antigens, it can also be concluded that the production of local antibodies is more specific and the cervix is the usual potential site for antibody production as suggested by Hulka and Omran (1969) and Omran and Hulka (1971) in human beings and bovines, respectively.

The results of immunological agglutination and their relationship with pregnancy is presented in Table 3. The low level of fertility observed in this experiment in reactors might be associated with agglutination and immobilisation of sperm, reduced sperm penetration, early embryonic death and failure of implantation (London *et al.*, 1984). It is thus quite logical to emphasis that the antibodies produced locally in the female reproductive tract have to be present until it reacts with specific antigen

causing early embryonic death (Kiddy *et al.*, 1959). Moreover the variation in pereentage of motile spermatozoa in female genital tract might have caused the difference in fertility rate in reactors (Wilson, 1954).

Among the non-reactors 10 (66.67%) and reactors 10 (27.03%) that conceived, might lead to conclusion that mild agglutination reaction observed in some animals might not have interfered with the establishment of pregnancy.

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Histo-pathological Investigations Of Genitalia In Repeat Breeder Cows

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ABSTRACT

The detailed examination of genital organs of 24 (10 C + 14 H) chronic repeat breeder cows and heifers were carried out on post-mortem to study the pathological conditions. The different gross pathological changes noted were hard enlarged cervix in 17, endometritis in 6, tubal affections in 14 and ovarian disorders in 4 animals.

The histo-pathological findings of different parts of the genital organs did not reveal a common pathological feature in all the animals studied. However, the different pathological conditions revealed were chronic cervicitis with varying degree of fibrous tissue proliferation in 10 cases, extensive oedema with compressed uterine glands in 4 cases, lymphocytic infiltration in 6 cases, cystic dilatation of uterine glands in 8 cases and chronic inflammatory changes of uterus in 5 cases. Fallopian tubes of 14 animals were affected and showed various pathological lesions like inflammatory changes, presence of cysts, desquamation of tubal epithelial lining and diffused thickening of epithelial lining.

* * *

Receptivity or hostility for the ascending or descending fertilized ovum is important consideration based on the uterine environment. Uterine conditions are studied these days with endometrial biopsy technique. Pathological changes in different parts of genital organs are responsible for repeat breeding or infertility. Inflammatory conditions of tubular genitalia lead to unfavourable uterine environment for fertilization of ovum and survival of fertilized zygote.

The pathological conditions of fallopian tubes were investigated by Azizuddin (1954) to determine their relationship to endometritis and repeat breeding. In his investigation, 32 out of 93 (29.31%) endometric uteri showed lesions in their fallopian tubes, while 2 cases showed primary infection of the fallopian tubes. Dawson (1956) investigated clinically and pathologically the condition of bursa and salpinx to study the incidence of inflammatory conditions in permanently sterile cows. It was found that 52.5 percent of the cows were affected with bursitis and endosalpingitis.

Under the present study a total of 24 repeat breeder cows and heifers were investigated for histo-pathological lesions in genital organs.

Materials and Methods

Twenty four cows and heifers which had not conceived after large number of inseminations (Average 12.5) were investigated on post-mortem for histo-pathological aspects of genitalia. The

Fig. 1: Proliferation of fibrous connective tissue in cervix. H & E ×100

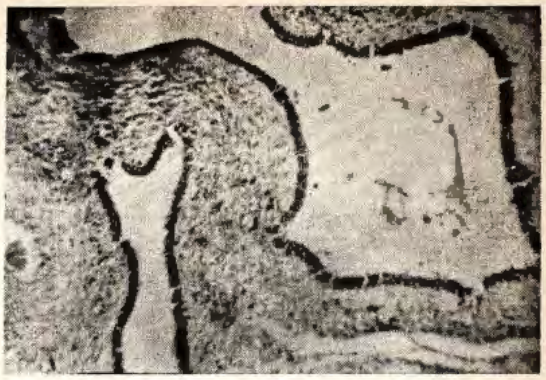

Fig. 4: Cystic dilatation of uterine glands. H & E × 400

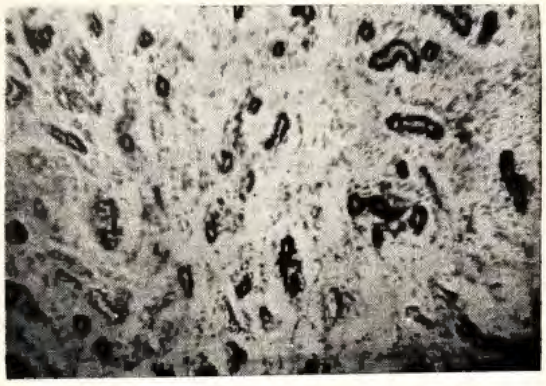

Fig. 2: Oedema of interglandular space with compression of uterine glands. H & E × 100

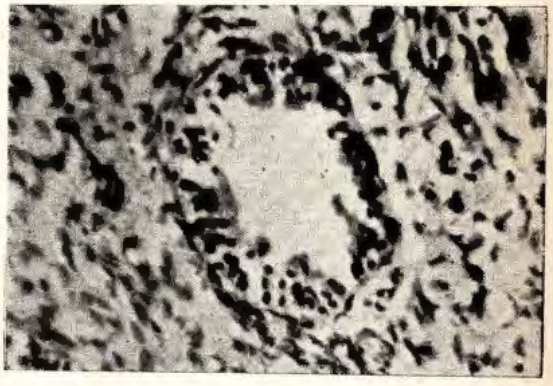

Fig. 5. Periglandular fibrosis in uterus. H & E×400

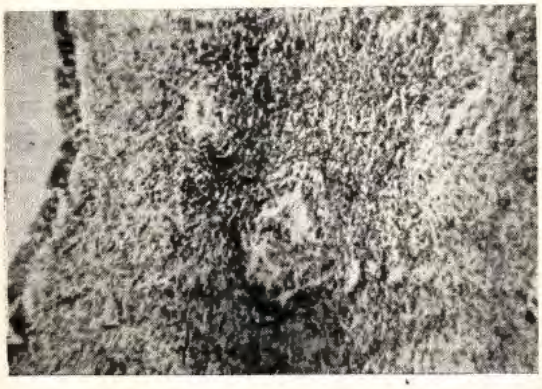

Fig. 3: Infiltration of lymphocytes in the endometrium with degenerating uterine glands. H&E× 100

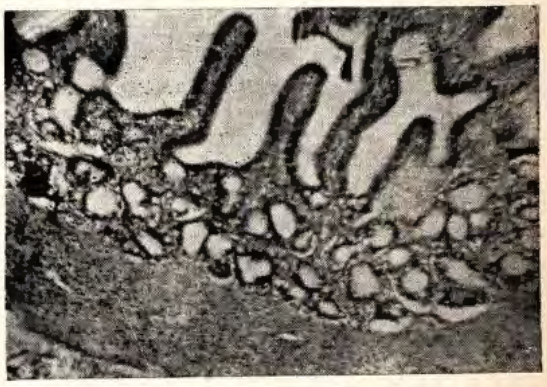

Fig. 6: Intra-mucosal cysts in the fallopian tube. H & E× 100

genital organs were collected on post-mortem examinations. Both ovaries, fallopian tubes, uterine horns (pieces) and cervix were preserved in 10 percent formalin for histo-pathological study. The tissues preserved were processed and sections of 4 to 5 μ were cut and stained with haematoxyline and eosin staining. All the slides were examined under microscope (10 and 45 X) for detection of pathological lesions.

Results and Discussion

The lesions observed were chronic cervictitis (10), various pathological lesions of uterus (19), fallopian tubes affections (14) and ovarian lesions (12).

These lesions were proliferation of fibrous connective tissue in cervix (Fig. 1), oedema of endometrium with compressed uterine glands (Fig. 2), focal lymphocytic infiltration forming aggregates (Fig. 3), cystic dilatation and degenerative changes in the uterine glands (Fig. 4), periglandular fibrosis and atrophy with inflammatory cells (Fig. 5) were observed in the uterus.

The fallopian tubes of 14 out of 24 animals showed pathological lesions. Major pathological alterations in the fallopian tubes observed were mucosal cysts (Fig. 6) in 4 cases, salpingitis in 4 cases (Fig. 7), papillary hyperplasia in 3 cases and hydrosalpinx in 3 cases (Fig. 8).

The lesions in the ovary included single or multiple cysts (Fig. 9) and unilateral hypoplasia in 1 case with absence of germinal epithelial cells.

The incidence of pathological lesions of the cervix observed in the present study was much higher than those reported in the literature (Lagerlof and Boyd, 1953). This might be due to too frequent breeding and animals remaining non-pregnant for a long period (18 to 24 months). Cervicitis was the most common pathological condition of the cervix in the present study and this was recorded in 10 cases (41.66%). There was no prominent lesion except extensive proliferation of fibrous connective tissue. It is possible that acute cervicitis developed first due to frequent breeding and later subsided leaving the segment as hard and indurated.

The common pathological lesions observed in majority of genitalia from the repeat breeders were moderate to extensive lymphocytic reaction in the endometrium with glandular dilatation, degeneration and periglandular fibrosis. Inflammatory lesions coupled with isolation of organisms from uterus showed mild or subclinical endometritis due to non-specific bacteria. The severity of lesions probably waned of due to repeated oestruses (Roberts, 1971). Cupps (1973), in a detailed study of the uterus of repeat breeder cows explained that endometritis consequent to trauma or infection evoked healing process in which the endometrial glands got blocked resulting in dilatation and fibrosis-ring around. He postulated that dilatation with periglandular fibrosis of endometrial glands resulted in infertility. Infiltration by lymphocytes and neutrophils resulted into infection, disturbed the normal uterine function and thus made nidation and survival of embryo impossible. Rao (1974) observed periglandular fibrosis, cystic dilatation of glands and lymphoid aggregates in lamina propria possibly due to low grade infection as the common lesion in repeat breeder Hariana cows. Present findings in repeating cows and heifers concurred with these views except that eosinophilic infiltration as observed by Cupps (1973) was not seen. In one heifer, uterine glands were scanty and under developed. The whole uterine wall

Fig. 7: Salpingitis — note the infiltration of mononuclear cells in mucosa of fallopian tube. H & E × 100

Fig. 8: Hydrosalpinx—note the flattened and atrophic villi. H & E × 400

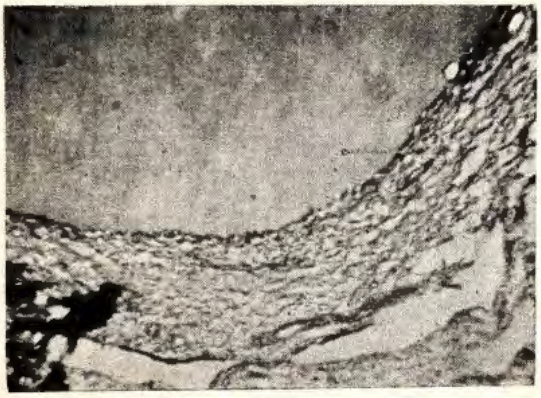

Fig. 9: Ovarian follicular cyst—note the complete disappearance of granulosa cells. H&E × 100

was thin and atrophied. Dawson (1963) in his studies on uterine pathology of 300 repeat breeding cows reported that in 27 cows, there was absence of uterine glands and surface epithelium.

The incidence of pathological conditions of the salpinx in the present study was similar to the earlier reports (Lombard *et al.*, 1951; Dawson, 1956 and 1958). The transport of sperms and ova depends upon cilliary action, muscular contraction and currents of fluid in the fallopian tube. Any pathological condition will alter the physiological functions of the tube (Karkun, 1974). Mild inflammatory lesions of salpinx are likely to go un-noticed as they do not bring about any palpable alteration in the size of the organ (Roberts, 1971).

Intra-mucosal cyst formed by the fusion of adjacent folds denuded from the ephithelial lining due to salpingitis have been reported (Lombard *et al.*, 1951; Roberts, 1971). All the cysts were of varying size. These may obstruct the tubal passage partially or completely depending upon size and number of the cysts present in the tubal mucosa.

The salpiugitis was observed in 4 (16.67%) cows out of 24 which is considerably a higher incidence. Moberg (1954) stated that descending infection from bursa or ovary as the cause of salpingitis, while Cembrowicz (1956) suggested ascending infection from the uterus as a cause. In 3 cases, salpingitis and excessively tortuous and hyperplasia condition of mucosal folds which coalesced with each other and obliteration of the lumen were the lesions that could account for tubal blockage. Dwivedi and Singh (1971) recorded the occurrence of microscopic multilocular cyst formation and salpingitis as the lesions that could account for tubal blockage in buffaloes.

It has been established that chronic salpingitis was the commonest basic cause for tubal blockage in cows (Dawson, 1964). Association of salpingeal lesions with uterine inflammatory lesions due to infections and cervicitis in 6 cases suggested that possibility of ascending infection as the basic cause of repeat breeding in the presently studied animals.

The repeating cows presently studied revealed ovarian lesions. The significance and association of follicular cysts small and large size (8 cases) and luteal cysts (3 cases) detected microscopically with repeat breeding conditions remained obscure. The cystic ovarian degeneration was high in present study. Garm (1949) reported multiple ovarian cysts to be of more frequent in occurrence than single cyst. However, in the present material, in 5 cases large single and in 3 cases small multiple cysts were observed. The study on cystic ovarian degeneration was also reported in Indian cattle by Goswami and Choudhary (1963) and Rao *et al.* (1965).

The ovarian lesions were suggestive of nymphomaniac conditions. Nymphomania has been defined by Garm (1949) with regular and irregular cycles. Clinical observations for the pattern of oestrus and cestrus cycles agreed with the above conditions.

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Fallopian Tube Patency Testing And Therapeutical Considerations In Repeat Breeder Bnffaloes

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ABSTRACT

Aero-insufflation test was carried out in the repeat breeder buffaloes by using specially designed "Insufflation Apparatus". In these animals the bilateral tubal patency, unilateral tubal blockage and bilateral tubal blockage were found to be 51.38, 29.16 and 19.14 percent, respectively. The rate of fall of infusion pressure was observed as rapid fall, gradual fall and maintained, in patent, unilateral blockage and bilateral blockage, respectively. The relationship between infusion pressure with percentage of animals showing patent tubes at each pressure group, has been studied. The maximum percentage (30.44%) of animals showed patent tubes at 50-99 mm Hg pressure.

In 72 repeat breeder buffaloes, Antibiotic solution was infused into uterus under pressure after tubal patency testing. These animals were inseminated in subsequent oestruses. The pregnancy diagnosis was carried out after 2 months by rectal examination. Among the total followed 58.92 percent buffaloes were found pregnant at mean fertile oestrus interval of 31.48±2.57 days. This treatment was found satisfactory for settling the repeat breeder buffaloes.

* * *

Fallopian tube is an important and vital link between the ovary and uterus. Tubal factors like endometritis, occlusion, aplasia and other lesions which are not gross and palpable could be responsible for repeat breeding. Roberts (1971) stated that the fallopian tube disorders and its diseases probably occurred more commonly than generally assumed and diagnosed. It is for this reason that the assessment of tubal patency has been generally considered as one of the essential test of infertility investigations.

Materials and Methods

Design of the instrument:

A new apparatus in partial modification of one designed by Chenne Gowda and Abdulla Khan (1975) was fabricated using indigenously available parts. The apparatus essentially consisted of the following units (Fig. 1).

1. A dial manometer (Japan) calibrated to read between 0 and 300 mm Hg pressure.
2. Rubber tubing (latex, 4 mm dia.) for assembling different parts for preparation of complete instrument.
3. Two 3 way 'T' shaped stainless steel valves.
4. Continuous pipetting 50 ml syringe.

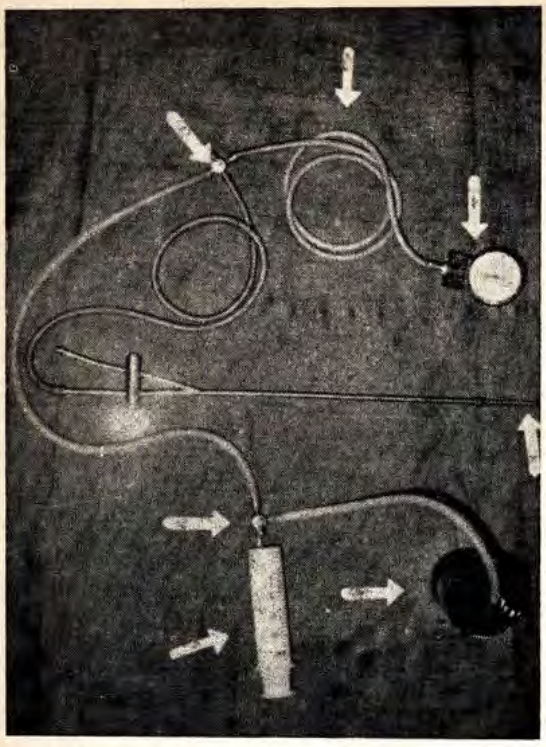

Fig. 1 Insufflation apparatus.

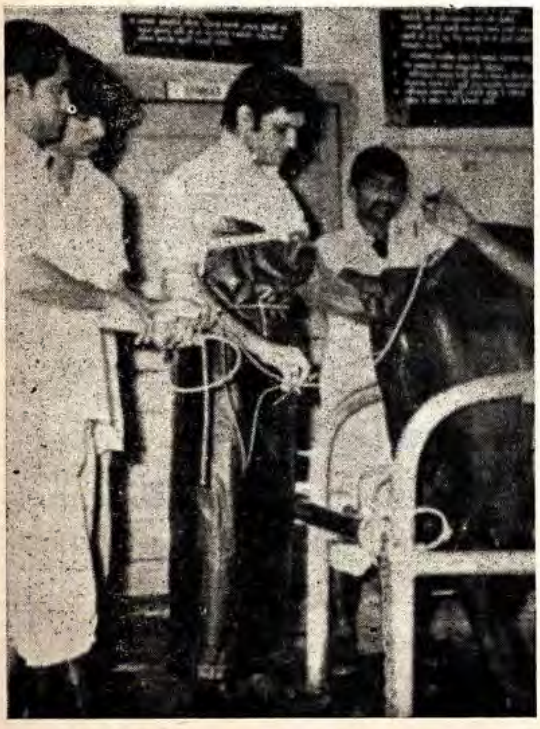

Fig. 2 Tubal patency testing.

5. Beaker containing antibiotic solution.
6. Stainless steel uterine cathetor 50 cm long & 5 mm in diameter with cervical stop to prevent back flow of air.

Procedure for tubal patency test (Fig. 2):

Animal to be tested was restrained properly in trevis. The vulva and perineal region was cleansed and dried. If the animal was not co-operative and struggled, epidural anaesthesia was induced. Rectal examination was carried out to know the reproductive status of the animal. Then, cervix was grasped and uterine cathetor was passed into the cervical canal upto the uterine body. The cervical stop was fixed on the external os to prevent the leakage or back flow of air. Air was infused into the uterus using the continuous pipetting syringe at the rate of 30 mm Hg pressure per minute. The gradual building up of pressure in the manometer was taken as an indication that there was no back flow of air from cervical canal. When sufficient pressure was built up, uterus was inflated, enlarged and air started escaping through ostium tubae abdominalis. If there was rapid fall in the intra-uterine pressure, this was considered indicative of tubal patency. The rate of fall in intra-uterine pressure was much slower in unilateral tubal impatency cases. On the other hand when intra-uterine pressure remained steady at 200 mm Hg for more than 3-5 minutes, these were considered as cases of bilateral impatency.

A total of 72 buffaloes which were chronic repeat breeders were tested by this method in the present study.

Treatment for tubal block:

On the study of excised genital organs Kavani *et al.* (1982) found that many fallopian tube blocks were temporary in nature. When such tubes tested with

TABLE 1. Tubal patency and rate of fall of pressure in repeat breeder buffaloes.

Rate of fall of pressure	Per cent	Tubal Patency
Rapid	51.38 (37)	Patent
Slow	29.16 (21)	Partial block or Unilateral impatency
Maintained	19.44 (14)	Bilateral blockage
Total	(72)	

Figures in parentheses indicate number of animals.

air-insufflation method showed blockages. But when fluid was infused from uterine end into the fallopian tube under pressure such temporary blocks got dislodged and the tube became patent. Taking this thing into consideration in repeat breeder buffaloes, the following treatment was designed and carried out.

After testing the animals by air-insufflation method, each animal was injected antibiotic solution intra-uterine under pressure ranging from 200-250 mm Hg. The antibiotic solution was prepared by dissolving 2.5 gm vial of Dicrysticin—S (Sarabhai Chem., Baroda) in 80-100 ml of distilled water. The infusion was done under 200-250 mm Hg pressure upto the demarcation and distension of uterus which was felt per-rectally. The same animal was inseminated during subsequent oestrus. The pregnancy diagnosis was carried out after 60 days of the last insemination. A total of 72 buffaloes were treated by this method and followed up for pregnancy results.

Results and Discussion

In the present study, 72 repeat breeder buffaloes were tested for tubal patency by air insufflation test. The bilateral tubal patency, unilateral impatency and bilateral impatency were 51.38, 29.16 and 19.14 percent, respectively. The details of rate of fall of pressure and tubal patency have been presented in Table 1.

On going through the published literature, it was found that very few studies have been reported on this aspects. Koike and Kawata (1959) observed 29.60 percent unilateral and 14.96 per cent bilateral impatency in excised genitalia of infertile cows. Further they

TABLE 2. Relation of infusion pressure with tubal patency in repeat breeder buffaloes.

Infusion pressure mm Hg.	Percentage tubal patency
0 to 49	15.22 (11)
50 to 99	30.44 (22)
100 to 149	22.22 (16)
150 to 199	12.50 (9)
200 & above	19.45 (14)*
Total	(72)

Figures in parentheses indicate number of animals
* Pressure was maintained.

TABLE 3. Average number of A.I. in relation to pressure in repeat breeder buffaloes.

Mean pressure mm Hg.	No. of animals	No. of A.I.
28.18 ± 4.45	11	6.27 ± 0.47
71.18 ± 2.75	22	5.95 ± 0.28
122.25 ± 3.63	16	7.38 ± 0.59
170.22 ± 5.74	9	9.88 ± 1.16
221.43 ± 4.45	14	13.36 ± 1.53

have reported a rapid fall in pressure when both the tubes were patent. Kelly *et al.* (1961) tested 69 excised genitalia of cows and out of them in 7 percent genitalia, tubal blockage was recorded.

Kavani *et al.* (1982) observed 15.98 percent unilateral and 4.34 percent bilateral tubal blocks in excised genitalia (438 organs) of Surti buffaloes. They further reported that the most frequent site of tubal block was the utero-tubal junction (72.50%), next in order were isthmus (22.25%) and ampulla (5.25%). Chenne Gowda and Abdulla Khan (1975) reported on tubal occlusions in excised genitalia when the pressure was beyond 300 mm Hg.

The amount of infusion pressure and the number and percentage of repeat breeder buffaloes falling in these infusion pressure groups were classified (Table 2). In 30.44 percent buffaloes, the fallopian tubes were found patent at 50-99 mm Hg pressure. Further, in repeat breeder buffaloes, the average number of services (A.I.) in relation to amount of pressure was classified. It was found that the relationship was of positive nature showing the amount of pressure increasing as the number of services increased. The detailed observations have been presented in Table 3.

From the present study it is apparent that rapid fall of pressure indicated patency, slow fall, partial or unilateral block and when pressure was maintained a bilateral block. Results under the present study clearly indicated the involvement of tubal factor as a major cause for the resultant chronic repeat breeding conditions in buffaloes. The methods adopted in the present study are easy and can be safely carried out under field conditions for infertility diagnosis. When the animal takes more number of Services/A.I. (≤ 4), it is an indirect indication that the tubal factors need to be tested.

Treatment for tubal blockage:

Considering the results obtained by flushing of fluid in fallopian tubes through

TABLE 4. Teatment of repeat breeder buffaloes with tubal flushing under pressure and pregnancy results.

Conditions	Number of animals treated	No. of animals followed	Pregnancy (%)	Fertile oestrus interval (days)
Bilateral patency	37	26	69.23 (18)	31.11 ± 3.59
Unilateral patency	21	18	55.55 (10)	30.09 ± 4.19
Bilateral impatency	14	12	41.66 (5)	34.00 ± 8.36

Figures in parenthesies sindicate number of buffaloes.

uterine end and dislodgement or dissolving of the temporary blocks, it was decided to infuse antibiotic solutions intra-uterine under pressure in repeat breeder buffaloes.

In all 72 buffaloes were treated with antibiotic infusion under pressure. Out of them 56 buffaloes could be followed for the results. Among the followed 33 (58.92%) buffaloes became pregnant at a mean fertile oestrus interval of 31.48± 2.57 days (Table 4). These results were very much encouraging. Tbe resultant pregnancies might be due to the removal of the tubal blocks in some and establishing the tubal patency. It is clear that considerable repeat breeder buffaloes have tubal impatency as a cause of repeat breeding. Hence, with due testing of the patency, treatment can be conventionally done in the field for settling the repeat breeder animals. Treatment in unilateral tubal obstruction was more encouraging than in the bilateral blocks. More work on this aspect is necessary on a large scale for tubal patency testing and antibiotic infusion treatment under pressure in field condition so that its efficacy can be fully known.

Acknowledgement

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Relationship Of Corpus Luteum Of Cycling Buffalo-Heifers With The Crystallization Pattern Of The Cervical Mucus And Its Sodium Content During Oestrus Cycle

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ABSTRACT

Studies were conducted on buffalo-heifers on the size of corpus luteum (CL), scores of crystallization pattern of cervical mucus (CM) and its sodium concentration in one complete oestrus cycle. The CL attained maximum size on day 12 of the cycle and exhibited regression in size from day 3 before oestrus and regressed completely by the day of the next oestrus. The scores of the crystallization pattern of the CM remained low during the entire diestrus period and increased with the regression of CL, attaining a maximum score on the day of oestrus. The concentration of sodium in CM though remained almost isotonic during the entire period of study, exhibited slight increase with the regression of CL and with increase in scores of the crystallization pattern of the CM.

* * *

Corpus luteum (CL) is known to play a major role in the length of oestrus cycle. Changes in cervical mucus (CM) occur during follicular phase and its disappearance during luteal phase has been reported by Gram and Skjerven (1952). Alliston *et al.* (1958) further elaborated that the first indication of appearance of fern in the CM becomes evident nearly 3.5 days before oestrus in cows and it reaches its height at the time of oestrus. Quyam and Vankatasami (1964) evaluated the ferning pattern and alloted values to them ranging from 5 to 95. Herzberg *et al.* (1964) reported that the sodium chloride content of fresh CM did not show cyclic variation and it was always isotonic. No report appears to be available on the correlation among these three parameters particularly in buffalo. The present paper embodies the relationship between the scores of crystallization pattern of CM, its sodium concentration and the size of the CL in buffalo-heifers during a normal oestrus cycle.

Materials and Methods

Eighteen cycling buffalo-heifers (Murrah breed) almost of the same age and body weight, maintained at the National Dairy Research Institute, Karnal, were used for this study. The ovaries were palpated as per the method described by Zemjanis (1970) and the size of CL was approximated. Samples of CM were collected as reported earlier by Parsad *et al.* (1980). Estimation of the size of the CL and collection of the CM were done daily (at fixed hour of the day) from day 0 of one cycle to day 0 of the next cycle (day 0=day of onset of oestrus) taking 21 days as the length of the oestrus

TABLE 1. Size of the corpus luteum of cycling buffalo-heifers, scores of crystallization pattern of cervical mucus and its sodium concentration (Mean ± SE).

Sr. No.	Days of oestrus cycle	Size of the CL (cm)	Scores of crystallization pattern of CM	Concentration of Sodium mg/100 g CM
1.	0	np(0)[a] (13)	96.68 ± 0.92[e] (13)	320.00 ± 6.12[f] (13)
2.	5	1.13 ± 0.10 (8)	15.00 ± 1.64 (8)	280.00 ± 09.11 (8)
3.	6	1.40 ± 0.09 (8)	23.13 ± 2.82 (8)	255.00 ± 10.64 (8)
4.	7	1.36 ± 0.10 (8)	27.14 + 2.40 (8)	261.00 ± 13.70 (8)
5.	8	1.45 ± 0.09 (7)	30.25 ± 2.10 (7)	260.00 ± 11.84 (7)
6.	9	1.61 ± 0.08 (7)	27.85 + 1.84 (7)	262.00 ± 13.35 (7)
7.	10	1.81 ± 0.10 (8)	26.85 ± 1.31 (8)	262.00 ± 07.80 (8)
8.	11	1.88 ± 0.08 (7)	35.00 ± 2.67 (7)	259.00 ± 11.84 (7)
9.	12	1.95 ± 0.09[b] (8)	29.37 ± 1.99[d] (8)	238.00 ± 9.50[g] (8)
10.	13	1.93 ± 0.06 (8)	30.00 ± 1.88 (8)	261.00 ± 6.91 (8)
11.	14	1.91 ± 0.08 (8)	30.00 ± 1.33 (8)	263.00 ± 7.31 (8)
12.	15	1.93 ± 0.10 (8)	30.00 ± 2.50 (8)	260.00 ± 9.37 (8)
13.	16	1.86 ± 0.07 (9)	39.96 ± 1.83 (9)	262.00 ± 7.68 (9)
14.	17	1.54 ± 0.10 (8)	52.40 ± 4.00 (8)	275.00 ± 9.05 (8)
15.	18	1.31 ± 0.10 (9)	65.00 ± 3.81 (9)	281.00 ± 7.15 (9)
16.	19	0.90 ± 0.11 (11)	80.90 ± 2.76 (11)	302.00 + 6.95 (11)
17.	20/0	np(0)[a] (13)	95.25 ± 1.10[e] (13)	318.00 ± 7.11[f] (13)

O = day of oestrus, np = non-palpable.
Figures in the parentheses indicate the number of observation.
Figures having common superscript under column do not differ significantly.

cycle. Animals returning to oestrus before and beyond 21 days were not included in this study. Evaluation of the crystallization pattern of the CM was carried out as described by Quyam and Venkatasami (1964) on the same day of sampling and the estimation of sodium concentration was made on later dates by Flame Photometer (Elco-model CL-22 at 589 mμ wave length), after digesting the samples according to the method described by Oser (1979) and the results were expressed in mg/100 g of CM. In between collection and analysis the samples were stored at —20°C to avoid loss of water. The study

on a particular date was based only on those animals from whom the CM could be collected. The differences between the values of these parameters at days, 0,12 and 20 were estimated by 't'-test (Snedecor and Cochran, 1968).

Results and Discussion

The size of corpus luteum, scores of crystallization pattern and the concentration of sodium in CM are presented in Table I.

Table I reveals that the CL on the day of oestrus was almost non-palpable. The size of fresh CL on days 1 and 2 were also non-palpable while on days 3 and 4 it was perceptible but the border remained undefined. Thereafter, the size of the CL gradually increased till day 12 and remained almost of the same size till day 16, after which it recorded regression in size and became hardly perceptible on the day prior to the next oestrus. It became non-plapable on the day of the next oestrus. Salisbury *et al.* (1978) also reported that the maximum size of the CL was attained on day 12, whereas, Zemjanis (1970) reported that the maximum size was attained on day 9 of the cycle in cows. The size of the CL in buffaloes reported by Fahimuddin (1975) was slightly smaller than that obtained in this study which may be because his study was based on slaughter house materials. The observation that the CL started regressing by day 3 before the onset of next oestrus is in agreement with the report of Roberts (1976) and Salisbury *et al.* (1978). The scores of the crystallization pattern of the CM was maximum on the day of oestrus. The scores remained low during the entire diestrus period. It increased with the regression of CL from day 3 before the oestrus and reached its maximum again on the day of oestrus when the CL was almost non-palpable. The result was in agreement with that of Quyam and Venkatasami (1964).

The concentration of sodium was 320.00 ± 6.12 mg/100 g of CM on the day of oestrus and it was slightly lower on days 5 to 16 of the cycle.

The value increased slightly on day 3 before the next oestrus and kept on increasing till the day of oestrus. Though the concentration of sodium in the fresh CM remained almost isotonic throughout the entire period of study, exhibited minor inverse change with the size of the CL and increased with the increase in the score of crystallization pattern of CM. Table I also reveals that the size of the CL, scores of crystallization pattern of CM and its sodium concentration on day 12 were significantly ($P<0.01$) different from those at day '0' and day 20. The difference between these estimates at days 0 and 20 were, however, statistically non-significant.

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Studies On Some Blood Constituents In Normal And Abnormal Cycling Buffaloes

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ABSTRACT

Investigation was done on 50 Murrah buffaloes to study blood constituents in normal and abnormal cycling buffaloes. The blood glucose level averaged 50.13 mg per cent in abnormal cycling buffaloes which increased to 66.65 mg per cent on day of induced heat. The pre and post treatment levels were found statistically significant ($P<0.05$). The serum calcium level in abnormal cycling and at oestrus after treatment averaged 13.2 and 13.39 mg per cent respectively with non-significant difference in silent heater and repeat breeder buffaloes. The inorganic phosphorus level in abnormal cycling and at oestrus after treatment averaged 6.46 and 6.83 mg per cent respectively with nonsignificant difference. The concentration of cholesterol in abnormal cycling and at oestrus after treatment averaged 54.11 and 51.63 mg per cent respectively. The pre and post treatment values were found significant between control and repeat breeder, anoestrus and silent heat groups ($P<0.05$). The haemoglobin percentage was 7.62 and 8.00 gm per cent in abnormal cycling and at oestrus after treatment respectively. The pre and post treatment differences were significant ($P<0.05$) except in repeat breeder buffaloes.

* * *

It is a well established fact that as productivity of milch animals rises one becomes more conscious of deficiency syndromes and metabolic disorders. It is quite possible that some of the blood constituents may be associated with abnormal cycling. Hence the present study was undertaken to ascertain the role of glucose, calcium, phosphorus, cholesterol and haemoglobin in normal and abnormal cycling buffaloes.

Materials and Methods

The investigation was conducted on 50 Murrah buffaloes belonging to the composite Livestock Farm of Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur as detailed below (Table 1):

Control group:

No treatment was given to the buffaloes of this group. Blood samples were collected on the day of gynaeco-clinical examination. Each animal of this group was observed for signs of heat upto 21 days. Blood samples were collected from the buffaloes that came in heat within the above period on the day of heat and also from the buffaloes that failed to come in heat within the above period, on day 21 of gynaeco-clinical examination.

Treatment groups:

Blood samples from all the buffaloes in these groups were collected just prior

TABLE 1: Group-wise details of the experimental buffaloes

Group No.	No. of buffaloes in the group	Condition of buffaloes	Type of group	Name of the group
I	10	Normal cycling	Control	Control
IIa	5	Anoestrus	Treatment	Fertivet
IIb	5	,,	,,	Fertivet with copper sulphate
IIc	5	,,	,,	Prajana
IId	5	,,	,,	Lugol's iodine with utero-ovarian massage
IIIa	5	Silent heater	,,	Prajana
IIIb	5	,,	,,	Lugol's iodine with utero-ovarian massage
IVa	5	Repeat breeder	,,	Mastalone-U
IVb	5	,,	,,	Gentamycin-sulphate

to treatment and after gynaeco-clinical examination. Each animal was observed for signs of heat upto 21 days. Blood was collected from the buffaloes coming in heat, following treatment on the day of induced heat.

Results and Discussion

In the present study the difference in blood glucose level and haemoglobin percentage was found non-significant between the values of normal cycling and on day of heat. The serum calcium, inorganic phosphorus and cholesterol levels were also found to vary nonsignificantly between the two stages.

In the present study the mean blood glucose level was higher on day of induced oestrus (69.42 mg %), on day of improved expression of heat (65.75 mg %) and at pregnancy (78.33 mg %) in repeat breeder buffaloes than 54.62, 55.3, 62.0 mg per cent during anoestrus, silent heat and repeat breeding respectively. The mean blood glucose level increased from 59.13 to 66.65 mg per cent on day of induced heat. Statistically the difference was highly significant ($P<0.01$). These findings are in partial agreement with Dhoble and Gupta (1979) who observed significantly lower blood glucose level during anoestrus as compared to cycling buffaloes. Low level of blood glucose in abnormal cycling may be an indication of subnormal energy status. Secretion of gonadotropins might have reduced or stopped due to hypothalamic failure to utilize glucose (McClure, 1965). Hafez (1969) reported that since FSH is a glycoprotein, carbohydrate portion is essential for the biological activity of hormones. Murty and Mullick (1960) and Bianca and Findlay (1962) reported increased glucose during summer. High temperature and summer stress activates sympatheticoadrenal system causing rise in the blood glucose.

The mean calcium concentration was lower during oestrus than during abnormal cycling. The difference was non-significant between pre and post-treatment serum levels. Similar were the observations of Samed *et al.* (1980). The lower

TABLE 2: Pre & post-treatment concentration of blood glucose, haemoglobin, serum calcium phosphorus and cholesterol in normal and abnormal cycling buffaloes

Condition of the animal	Haemoglobin(g%)		Glucose(mg%)		Calcium(mg%)		Phosphorus(mg%)		Cholesterol(mg%)	
	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average
Normal cycling	7.8—9.4	7.29	62.50—90.00	79.75	10.00—15.00	11.80	4.5—8.0	5.95	40.23—74.50	59.00
On day of heat	8.0—8.4	8.2	72.50—87.50	80.16	11.00—13.00	12.00	6.0—7.0	6.50	41.72—61.09	52.64
Anoestrus	6.6—8.6	7.54	42.50—82.50	54.62	9.00—18.00	14.15	5.9—9.5	6.56	50.54—131.12—	73.02
On day of heat	6.6—8.6	7.78	45.90—100.00	69.42	9.00—1.60	12.62	5.5—9.5	6.87	44.70—77.48	60.57
Silent heat	7.0—9.0	7.98	32.50—70.00	55.30	11.00—16.00	12.80	4.5—10.0	6.25	53.64—131.12	69.88
On day of improved heat	7.0—10.0	8.45	57.50—82.50	65.75	11.00—14.00	12.62	6.0—8.5	6.76	52.15—65.56	57.92
Repeat breeder	7.0—8.6	8.06	52.50—82.50	62.00	9.00—16.00	12.40	5.5—8.0	6.60	52.15—73.01	61.53
At conception	7.6—8.2	7.88	70.00—90.00	78.33	15.00—10.00	16.41	4.5—6.5	5.20	34.27—47.68	40.97

serum calcium level during oestrus might be attributed to the high oestrogen titre at oestrus which induces hypocalcaemia (Laing 1965). Morrow (1969) reported that calcium deficiency did not affect reproductive performance of the cows.

The role of phosphorus in regulation of oestrus rhythm is well established. Sane (1977) reported that with phosphorus deficient rations in cows the symptoms of oestrus were supressed to such an extent that even with vasectomised bull, heat detection was difficult. Similarly Bhaskaran and Abdulla Khan (1981) reported that marginal deficiency of phosphorus is sufficient to cause disturbance in pituitary ovarian axis without manifestation of specific deficiency symptoms. The mean serum inorganic phosphorus level increased after treatment in abnormal cycling buffaloes on the day of oestrus from 6.46 to 6.83 mg per cent. However, the difference was non-significant. This finding is in partial agreement with Gupta (1977) and Shrivastava *et al.* (1982).

There exists a correlation between the gonadal steroids and cholesterol metablolism (Robinson, 1957). Cholesterol is found to be the precursor of progesterone. Therefore, its level increases in anoestrus or pregnancy. The blood cholesterol level decreases when animals are exposed to direct sun rays as the physiologic phenomenon for its destruction (Hafez *et al.*, 1958). In the present study the cholesterol level was higher as 69.36 mg per cent during abnormal cycling which declined to 54.97 mg per cent when animal came in heat. This finding is in line with Zala *et al.* (1972) and Luktuke *et al.* (1977) who reported higher cholesterol levels during anoestrus and lower during proestrus and oestrus as during anoestrus it is not adequately utilized for production of gonadotropins. The pre and post treat-

ment blood cholesterol levels were found statistically non-significant in anoestrus and silent heater buffaloes but it was significant ($P<0.05$) in repeat breeder buffaloes when they became pregnant after treatment. The cholesterol required for the synthesis of hormone might be too less to significantly influence the post treatment blood concentration.

In the present study the mean haemoglobin percentage raised from 7.67 to 8.0 g per cent in abnormal cycling buffaloes when they came in heat after treatment. The difference was found statistically significant amongst all the groups. These findings are in line with Gupta (1977) and Dhoble and Gupta (1981). However the variation in the haemoglobin concentration in abnormal cycling buffaloes might be due to the effect of environment, temperature and humidity as reported by Mullick (1960) and Pandey and Roy (1969a).

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Blood Serum Protein At Different Stages Of Pregnancy In Surti Buffalo

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ABSTRACT

Blood serum protein was estimated from seven Surti breed buffaloes for entire gestation period (Total 35 stages) over three parities. Overall average was 8.78 g/100 ml for pregnant buffaloes which was significantly higher than open cycling buffaloes. A positive correlation between protein concentration and stage of pregnancy was noticed upto 275th day of gestation. Towards the term, specifically 15th day antepartum it started decreasing reaching the lowest at 6 hrs prior to parturition. As the parity increased level decreased, and difference between lactating pregnant and non-lactating pregnant groups was significant.

* * *

During pregnancy, protein as a nutrient is an essential component for both the dam and the growing foetus (West and Todd, 1967). Available literature gives very little information on this aspect specifically for entire gestation, hence an attempt was made to know the levels of protein in Surti buffaloes for different stages of entire gestation (fertile heat through parturition : 35 stages).

Materials and Methods

Present investigation included three successive pregnancies of seven normal Surti buffaloes. The animals were maintained on the university farm under standard practices of feeding and management throughout the period of study. Blood sampling was done from jugular vein over 35 stages of entire gestation. Frequency of blood collection was high during early and late gestation including last 24 hrs (Fig. 1).

Blood serum was analysed for total protein by the method of Lowry *et al.* (1954). Complete data were analysed statistically for stage variation. The data were grouped into seven phases (Table-1) and analysed by factorial design as per Steel and Torrie (1960).

Results

Average protein levels for 35 stages have been represented in the form of graph (Fig. 1) and analysis of variance is given in Table-2.

Concentration of protein on the day of fertile heat was 7.87 ± 0.37 g/100 ml which increased non-significantly to 8.93 ± 0.24 g/100 ml on the second day. Thereafter it oscillated non-significantly till day-65 (Fig. 1). From day-65 onwards protein concentration had a specific increasing trend which continued till last 15th day of gestation. Peak level of 9.70 ± 1.00 g/100 ml was recorded for day-245th and day 260th.

Last 15 days of gestation showed decreasing tendency which continued upto

TABLE 1: Phase—wise serum total protien content

phase	1	2	3	4	5	6	7
Mean Protein (g/100ml)	7.86	8.49	8.47	8.87	9.37	9.24	7.90
±SE	±0.36	±0.40	±0.31	±0.42	±0.42	±0.71	±1.25

Details of Phases

Phase I	Fertile heat
Phase II	Day — 2 to Day — 10 (Period of fertilization, shedding of zona pellucida and nidation).
Phase III	Day — 15 to Day — 50 (Period of implantation and chorion development)
Phase IV	Day — 65 to Day — 125 (Period of foetation and organogenesis)
Phase V	Day — 155 to Day — 275 (Period of mid gestation and foetal development)
Phase VI	Day 15 antepartum to the day of parturition. (Period of udder enlargement, teat engorgement, labour pain and initiation of parturition).
Phase VII	Post-partum stage.

6 hrs antepartum. Last two stages showed little increase in the estimate. However the levels were quite low compared to peak levels (Fig. 1). The estimate at 6 hrs antepartum was significantly low ($P<0.05$). Immediately after parturition it decreased. As the parity increased, the level decreased. For the first, second and third parity, the mean serum protein concentration was 9.18, 8.24 and 8.25 g/100 ml, respectively.

Phase-wise analysis revealed that highest concentration was recorded for phase 5 followed by phase 6, and lowest was at phase 7 (Table 1).

Discussion

Growing foetus draws nutrients and raw materials from dam's circulation which is also a source of gamma globulins (Smith, 1962). Total protein in the circulation represents the balance between the biosynthesis and catabolism or mechanical loss. A variety of changes in the concentration of plasma protein have been observed during pregnancy because protein metabolism is put under an extremely severe stress during the development of foetus (Kaneko and Corneleous, 1970)

Results of present study gave an average level of protein as 8.78 g/100 ml for pregnant animals which was higher as compared to non-pregnant cycling heifers (3.45 g/100 ml, Anon., 1976). Work of Memon and Mullick (1961) for buffalo and Murtuza (1977) for cattle also showed same type of results, nevertheless their study was restricted to certain stages of pregnancy only.

As the pregnancy advanced, protein concentration in maternal circulation increased upto 15 days antepartum (Fig. 1) indicating a positive association with pregnancy establishment. The fluctuation (ups & downs) upto day-65 were specific which probably reveals its demand and utilization for the physiological events that take place during this period (fertilization, release of blastocyst

from zona pellucida, uterine preparation, chorion development and placentation, McDonald, 1980).

The estimate after day-65 showed a considerable increase which continued upto day 15 antepartum (Fig. 1). The rise noticed between the period day 185 to day 260 was significant ($P<0.05$). Increase in the protein concentration during mid gestation period may be explained as increased demand of protein by growing foetus and that for late gestation may be due to increase synthesis of globulin fraction essential for colostrum synthesis (Larson, 1958). Results of Reigle and Nellor (1966) and Roubicek and Ray (1972) for exotic cattle were also in agreement.

TABLE 2:

Analysis of variance

Sources	*df*	*ET*	*mrs.*
a Animal (A)	6	*g*	2.51
b Phase (Ph)	6	*d*	7.25**
c Perity (P)	2	*e*	13.20**
d A × Ph	36	*g*	1.23
e A × P	12	*g*	1.80
f Ph × P	12	*g*	7.73**
g A×Ph×P	72		1.28

*P< 0'05 **P< 0.01

ET = Error Term.

Towards the late gestation, specifically last few days the estimate was decreasing (Fig. 1). Same type of results have been reported by Larson (1958), Mc Lennan and Willoghby (1975), Blaurmel and Kruger (1976), Rowland

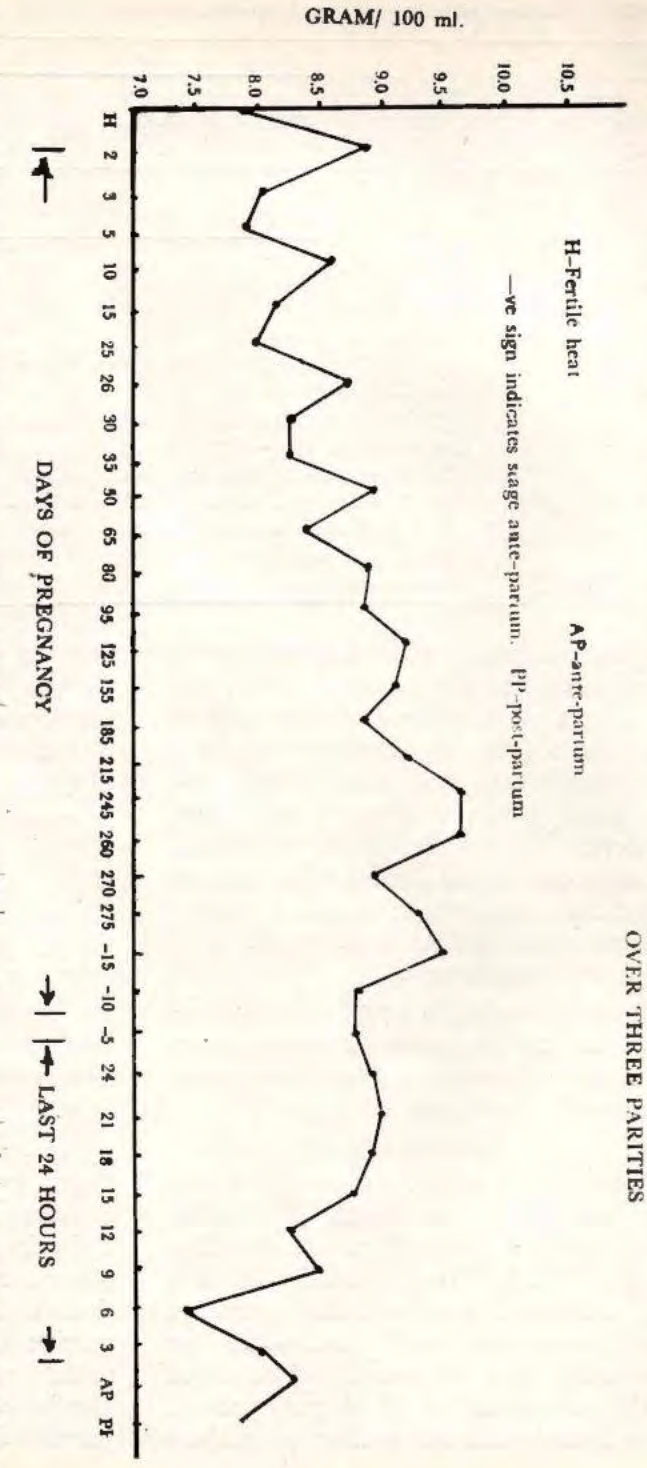

FIG. 1. SERUM TOTAL PROTEIN (GM. 100 ml) DURING DIFFERENT STAGES OF PREGNANCY OVER THREE PARITIES

et al. (1975) and Savoiskii and Federov (1976) for dairy cattle. As per Smith (1962), 10 to 30% decrease in blood serum protein occurs during 4 weeks prior to calving in cattle. From the results of present investigation it can be stated that in buffaloes probably it may be so within 10 days prior to calving. However, a detailed study for the same is needed.

The behaviour of protein for last 24 hrs was remarkable, the estimate decreased highly significantly ($P<0.01$) at 6 hrs antepartum and increased a little towards the parturition and decreased after parturition. This indicates that highest degree of transfer of glabulin to mammary gland in buffaloes may be taking place around 9 to 6 hrs antepartum.

Protein concentration for second and third parity was significantly low ($P< 0.05$) as compared to that of first parity. This is due to the fact that animals of second and third parity were lactating pregnant while that of first parity were dry pregnant.

No correlation was observed between the total protein level of maternal circulation and length of gestation and sex of the calf born.

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Dry Matter, pH, Lipid And Cholesterol Levels Of Cervico-Vaginal Mucus As Index Of Heat And Early Pregnancy In Catte

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ABSTRACT

The dry matter, total lipids and cholesterol contents of cervico—vaginal mucus of Jersey cows showed cyclic variations during different stages of oestrous cycle, whereas the pH showed an erratic trend. The values were found to be higher during the non-oestrous phase as compared to oestrous phase both in pregnant as well as in nonpregnant animals in case of dry matter, total lipids and cholesterol but the trend was not well defined in case of pH. The dry matter and pH levels did not show any significant difference during different stages of oestrous cycle of pregnant and non-pregnant groups but significant differences were recorded in the levels of total lipid and cholesterol contents during non-oestrous phase of pregnant and non-pregnant animals. It was concluded that the last two parameters can form a basis of diagnosing early pregnancy in cattle, a level of 75.4 mg total lipids and 5.35 mg of cholesterol per 100 g of mucus (wet wt.) indicating a positive state of pregnancy at 21 days.

* * *

Much work has been done on the morphological studies and physical characteristics of cervico-vaginal mucus in cows but little has been said about the biochemical changes in this mucus during different stages of oestrous cycle. The present investigation was, therefore, planned to obtain some basic information regarding dry matter, pH, total lipid and cholesterol content of genital secretions (mainly cervico-vaginal mucus) and to explore out the possibilities if any, that, could these parameters form a basis for developing a biochemical test for detecting accurate stage of oestrus and diagnosing early pregnancy in cattle.

Materials and Methods

The present study was conducted on 20 healthy and normally cycling Jersey cows of Indo-New Zealand Livestock Improvement Project of Himachal Pradesh Krishi Vishva Vidyalaya, Palampur. Samples of cervico-vaginal mucus were collected during early heat (EH, first 4-6 hr), mid-heat (MH, next 10-12 hr) and late heat (LH, next 4-6 hr till cessation of heat) during oestrous phase and after 1, 3, 6, 9, 12, 15, 18, 21, 24, 27, 29 and 30 days of heat during non-oestrous phase of the cycle by the use of sterilised cotton-swab technique (Arya & Jain, 1985). Dry matter was determined gravimetrically and pH on a digital pH meter. Lipids were extracted from the mucus samples with chloroform-methanol

TABLE 1: Dry matter percentage and pH of cervico-vaginal mucus of Jersey cows during different stages of oestrous cycle in pregnant and nonpregnant groups.

Phase	Stage	Dry matter (%)		Sig.	pH		Sig.
		Pregnant group	Non-preg. group		Pregnant group	Non-preg group	
		$\bar{X} \pm SE$	$\bar{X} \pm SE$	(P<0·05)	$\bar{X} \pm SE$	$\bar{X} \pm SE$	(P<0·05)
Oestrous	EH	2·43±0·36	2.25±0.24	NS	8.25±0.10	8.08±0.10	NS
	MH	1.28±0.10	1.59±0.20	NS	8.24±0.05	8.14±0.10	NS
	LH	2.03±0.41	1.74±0.27	NS	8.38±0.10	8.13±0.17	NS
	$\bar{X} \pm SE$	1.91±0.14	1.86±0.12	NS	8.29±0.04	8.12±0.02	NS
Non-oestrous Day	1	2.72±0.41	2.77±0.98	NS	8.17±0.10	7.86±0.14	NS
	3	4.00±0.09	3.59±0.99	NS	7.93±0.14	8.04±0.10	NS
	6	5.07±0.79	4.91±0.71	NS	7.98±0.14	7.91±0.05	NS
	9	4.99±0.69	6.90±1.44	NS	7.91±0.14	7.59±0.10	NS
	12	4.69±0.63	6.76±1.39	NS	7.98±0.10	7.63±0.14	NS
	15	5.51±0.96	7.33±1.78	NS	7.79±0.14	8.04±0.10	NS
	18	5.91±0.91	6.51±1.83	NS	7.92±0.14	8.03±0.17	NS
	21	5.98±0.85	7.19±1.92	NS	7.95±0.14	8.08±0.07	NS
	24	6.0±0.77	3.97±0.43	NS	7.93±0.14	8.43±0.10	NS
	27	7.93±1.19	4.13±1.44	NS	7.91±0.14	8.16±0.10	NS
	29	9.73±2.15	4.00±0.24	NS	7.93±0.10	8.35±0.55	NS
	30	10.09±2.17	9.03±0.04	NS	8.04±0.10	8.00±0.10	NS
	$\bar{X} \pm SE$	6.05±0.63	5.59±0.56	NS	7.95±0.05	8.01±0.10	NS

(2:1, v/v) in accordance with the method of Folch *et al.* (1957). Cholesterol was determined in the lipid samples using Lieberman-Burchard reaction (Cook, 1958). Each animal was inseminated artificially towards the end of late heat (LH) and was considered to be pregnant on 30 days non-return basis. Pregnancy was further confirmed by rectal palpation of foetus after a preiod of 3 months and from actual calving records. Out of 20 animals studied, 12 were found to be pregnant and 8 non-pregnant.

Results and Discussion

The dry matter, total lipids and cholesterol contents of cervico-vaginal mucus of Jersey cows showed cyclic variations during different stages of oestrous cycle whereas pH showed an erratic trend. The average values of dry matter (%) varied from 1.00 to 3.02 with a mean value of 1.92±0.14 during oestrous phase and from 3.64 to 10.78 with a mean of 6.05±0.56 during non-oestrous phase in pregnant animals. The corresponding values (with mean in parenthesis) for non-pregnant animals were 1.36 to 2.34 (1.36±0.12) and 2.55 to 10.87 (5.52±0.94), respectively. The values were lowest during mid-heat and started rising with advancement of cycle/pregnancy (Table 1). Our findings are in line with those of Noonan *et al.* (1957). This increase in dry matter content with the advancement of pregnancy can be attributed to increased levels of progesterone and decreased oestrogen levels (Linford, 1974). As the progesterone dominates, the mucus contains more of cells and leucocytes which

TABLE 2: Total lipid and cholesterol content (mg/100 g, wet wt) of cervico-vaginal mucus of Jersey cows during different stages of oestrous cycle in pregnant and nonpregnant groups.

Phase	Stage	Total lipids		Sig.	Cholesterol		Sig.
		Pregnant group $\bar{X}\pm SE$	Non.preg.group $\bar{X}\pm SE$	($\rho<0.05$)	Pregnant group $\bar{X}\pm SE$	Non-preg.group $\bar{X}\pm SE$	($\rho<0.05$)
Oestrous	EH	47.9±0.9	47.4±0.5	NS	0.189±0.001	0.184±0.002	NS
	MH	49.8±0.8	49.3±0.7	NS	0.096±0.002	0.095±0.003	NS
	LH	52.3±0.7	51.9±0.9	NS	0.053±0.001	0.050±0.001	NS
	$\bar{X}\pm SE$	50.0±1.3	49.5±1.3	NS	0.113±0.040	0.110±0.040	NS
Non-oestrous	Day 1	38.6±0.7	36.9±0.7	NS	0.976±0.003	0.972±0.001	NS
	3	92.0±1.3	89.4±0.9	NS	6.752±0.002	6.754±0.010	NS
	6	53.3±0.7	51.3±0.8	NS	2.017±0.008	1.998±0.002	NS
	9	57.6±0.5	56.1±1.2	NS	2.423±0.002	2.419±0.002	NS
	12	60.8±0.8	58.8±1.0	NS	3.054±0.002	3.049±0.001	NS
	15	66.2±1.1	63.6±0.9	NS	3.854±0.002	3.849±0.007	NS
	18	70.3±1.1	61.4±3.9	S	4.516±0.002	4.514±0.003	NS
	21	75.4±1.2	62.4±5.8	S	5.346±0.002	3.447±0.572	S
	24	80.0±0.9	70.3±7.2	S	6.167±0.002	4.960±0.818	S
	27	84.3±0.8	69.7±10.9	S	6.827±0.003	5.000±1.413	S
	29	88.3±0.5	84.5±1.5	S	7.820±0.017	6.721±0.093	S
	30	92.3±0.5	88.5±1.5	S	8.532±0.002	7.712±0.021	S
	$\bar{X}\pm SE$	71.6±4.9	66.1±4.5	S	4.857±0.702	4.283±0.596	S

further contribute to increase in dry matter content (Laing, 1970). The difference in dry matter content during different stages of oestrous cycle of pregnant and non-pregnant groups were statistically non-significant (Table 1), hence this parameter can not be used as diagnostic tool for pregnancy. However, the difference in dry matter during oestrous and non-oestrous phases of respective groups were statistically significant, hence this parameter can be used for positive and accurate detection of oestrous.

The cervico-vaginal mucus of the experimental cows was predominantly alkaline during different stages of oestrous cycle. These observations are in conformity with those of Roark and Herman (1950) and El-Naggar and Baksai-Horvath (1972). In general, the pH values were higher in pregnant as compared to non-pregnant group during oestrous phase but the trend was not well defined during non-oestrous phase (Table 1). Since the values were mostly overlapping and the differences were non-significant (Table 1) during oestrous as well as non-oestrous phase of both pregnant and non-pregnant groups, this parameter can not form a basis for accurate detection of heat or early pregnancy in Jersey cows.

The average values of total lipids (mg/100 g, wet wt) varied from 46.3 to 55.3 with a mean value of 50.0±1.3 during oestrous phase and from 66.7 to 74.7 with a mean of 71.6±4.9 during non-oestrous phase in pregnant animals. The corresponding values (with mean in parenthesis) for non-pregnant animals were 47.7 to 53.0 (49.5±1.3) and 56.1 to 72.2 (61.1±2.0), respectively. The average cholesterol content (mg/100 g,

wet tw, with mean in parenthesis) ranged from 0.108 to 0.120 (0.113±0.001) during oestrous and 4.849 to 4.865 (4.857±0.002) during non-oestrous phase in pregnant animals with corresponding values in non-pregnant animals as 0.105 to 0.114 (0.110±0.004) and 3.214 to 4.604 (3.617±0.209), respectively. The values were found to be higher in the non-oestrous phase as compared to oestrons phase both in pregnant as well as in non-pregnant animals. Cyclic changes in the ether extract of bovine cervical mucus with higher values in dioestrus were also reported by Boyland (1946). The values of total lipid content recorded in this investigation were higher than those reported by Wani *et al.* (1979). But our values of cholesterol were much lower than the values reported by them.

A comparison of lipid and cholesterol levels in the oestrous and non-oestrous phases of the pregnant and the non-pregnant animals (Table 2) revealed that statistically significant difference occurred in the oestrous and non-oestrous phases of the respective groups (50.0±1.3 Vs 71.6±4.9 in pregnant and 49.5±1.3 Vs 61.1±2.0 in nonpregnant in case of lipids and 0.113±0.001 Vs 4.857±0.002 and 0.110±0.004 Vs 3.617±0.209 in case of cholesterol, respectively). This suggests that these parameters can form a basis of detecting oestrous positively and accurtely. A further comparison of these components in pregnant and nonpragnant groups indicated that the pregnant animals contained higher lipids (71.6±4.9 Vs 66.1±4.5) and cholesterol (4.857±0.702 Vs 4.283±0.596) contents than nonpregnant animals during non-oestrous phase. The difference was discernible from the very first day but it was statistically significant on day 18, 21, 24, 27, 29 and 30 in case of total lipids and on day 21, 24, 27, 29 and 30 in case of cholesterol content. These parameters can, therefore, be used successfully for diagnosing early pregnancy after 21 days onwards.

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Hormonal Induction Of Ovulation And Subsequent Fertility In Adult Cycling Ewes

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ABSTRACT

Adult cycling ewes of Nali breed were treated with LHRH and Progesterone + PMSG to see their effects on ovulation rate, oestrous behaviour and fertility. 4 ewes out of 12 from the control group were laparotomised and the remaining 8 were subjected to natural breeding. The second group of 12 ewes was treated with 300 µg of LHRH on Day 16 of the oestrous cycle and 4 were laparotomised and 8 were bred. In the third group, 8 were treated with 10 mg. progesterone daily for 14 days and 1000 I.U. of PMSG on progesterone withdrawal and four ewes were laparotomised and four naturally bred. In the fourth group, 8 ewes were treated with 25 mg. progesterone on Day 0, 7 and 14 of the oestrous cycle and 750 I.U. PMSG was administered on Day 7 or 14 with four laparotomised and four bred. A repeat trial was conducted in group 3 with 12 ewes each getting 1000 or 1500 I.U. PMSG and 6 ewes were laparotomised and all the 12 ewes in this group were naturally bred including the laparotomised ones. There was suppression of overt oestrus in all the treated groups, the maximum suppression being observed in group 3. Ovulation was 100% in the progesterone treated groups (3 and 4) and 81.8% in the LHRH treated group but lambing was poor, being zero in group 3 and 50% in group 4 against 62% in group 2 and 66.7 in group 1. Oestrus duration and cycle length was decreased in LHRH treated group and increased in progesterone treated group in comparison to control. Ovulation time from onset of oestrus was 47 and 51 hours respectively in group 3 and 4 in comparison to smaller values of 28 hours in group 1 and 2. Superovulation was not detected in any of the treated groups.

* * *

Use of synthetic preparation of LHRH has revealed that it will induce release of LH in anoestrus (Reeves *et al.*, 1972), cycling (Schally *et al.*, 1973) and immature (Rippel *et al.*, 1972) ewes. Segerson *et al.* (1974) studied the effects of LRF in progesterone primed cycling ewes. The results indicated that although ovulation can be induced by LRF, fertility of released ova may be impaired if ovulation is induced prematurely.

Augmentation of fertility in cycling ewes with the use of progesterone and PMSG therapy has been achieved with success (Gordon, 1963). Sengupta *et al.* (1976) reported that a dose of 750 I.U. PMSG was satisfactory in respect of superovulation, conception rate and lambing performance of ewes. Pandey *et al.* (1972) reported better multiple births and lamb per ewe in multiparous

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TABLE: 1 Experimental design for induction of ovulation in adult cycling ewes.

Age 2½ — 5 years.
Av. body wt. 25.9 kg.

GROUP 1 CONTROL		GROUP 2 LHRH		GROUP 3 PROGESTERONE + PMSG		GROUP 4 PROGESTERONE + PMSG	
(12)		(12)		(8)		(8)	
LAPRO (4)	BRED (8)	LAPRO (4)	BRED (8)	LAPRO (4)	BRED (4)	LAPRO (4)	BRED (4)

Figures in parentheses indicate number of ewes.

TABLE 2: Schedule of hormone administration in adult cycling ewes.

Group	Hormone	No. of ewes		Dose	Vehicle	Route	Laparotomy interval	Slaughter interval	Oestrus detection
		Laparo-tomy	Bred						
1	Control	4	8	—	1.5 ml NSS	I/V	30-36 hr. from onset of oestrus	96-120 hr. after treatment	8 hourly from treatment till 24 hr. & 4 hourly until end of heat.
2	LHRH	4	8	300 µg	1.5 ml. NSS	I/V	— do —	— do —	— do—
3	P			10 mg. daily for 14 days.	PG	I/M			
	PMSG	4	4	1000 I.U. after P withdrawal	NSS	S/C	— do —	Within 8 days after PMSG administration	12 hourly from treatment till 24 hr. & 4 hourly till end of heat.
4	P			25 mg. on days 0, 7 & 14	PG	I/M			
	PMSG	4	4	750 I.U. on 7th or 14th days.	NSS	S/C	— do —	— do —	— do —
Repeat (same as group 3)		6	12*	As in group 3 (PMSG dose was 1000 & 1500 I.U. in repeat group)					

P, Progesterone; PG, Propylene glycol; NSS, Normal Saline Solution.
* All 12 ewes including laparotomised ones were used for breeding.

Magra ewes treated with 750 I.U. of PMSG. Honmode (1971), found that oestrus synchronisation and multiple ovulation were satisfactory but lambing performance was poor in ewes treated with progesterone and PMSG. The present investigation was carried out to study the effects of LHRH and progesterone+PMSG on ovulation, oestrus behaviour and fertility of adult cycling Nali ewes.

Materials and Methods

Fourty adult cycling female (age 2½ to 5 years) were included in this study. The regularity of their cycle was confirmed

TABLE 3: Hormone induced oestrus behaviour in cycling ewes.

Group	Treatment	No. of ewes	Overt oestrus	
			No.	%
1	Control	4	4	100.0
2	LHRH	12	4	33.3
3	Progesterone (10 mg.) + PMSG	8	1	12.5
4.	Progesterone (25 mg.) + PMSG	8	3	37.7
Repeat Expt.				
3	Progesterone (10 mg.) + PMSG	12	4	33.3

by preliminary observation on oestrus behaviour for over two months. For groups 1 and 2, Day 16th of the cycle as determined by the previous record of oestrus detection, was ascertained: These ewes were then sub-grouped so as to receive respective hormone treatment on Day 16 of their cycle. For groups 3 and 4, cycling ewes were selected for treatment irrespective of day of cycle and sub-grouped. The experiment of group 3 was repeated on 12 ewes during same breeding season & in similar condition.

Experimental design and schedule of drug administration is given in Table 1 and Table 2 respectively. The experiment of group 3 was repeated including 12 ewes.

Hormones:

LHRH was obtained from National Institute of Health, Abott (Lot 26-306 AL) and was dissolved in Normal Saline Solution (NSS) so as to contain 200 µg LHRH per ml

Progesterone:

Pregnen (4) dion-beta 20 was dissolved in propylene glycol to contain 10 mg. per ml. and was kept in refrigerator for daily use.

TABLE 4: Hormone induced ovulatory responses observed on laparotomy in cycling ewes.

Group	Treatment	No. of ewes laparo-tomized	Ovulation		No. of ovulations detected	
			No. of ewes	%	Right ovary	Left ovary
1	Control	1	1	100.00	—	1
2	LHRH	11	9	81.81	4	5
3.	Progesterone (10 mg.) + PMSG	3	3	100.00	2	1
4	Progesterone (25 mg.) + PMSG	2	2	100.00	2	—
Repeat Expt.						
3.	Progesterone (10 mg.) + PMSG	6	2	33.20	1	2

Note: No superovulation was detected in any of the treated ewes.

PMSG:

Freeze dried crystalline salt of PMSG was obtained from ICN pharmaceuticals Inc. (Lot OIDO), Cleveland U.S.A. and was dissolved in NSS to contain 750 I.U. per ml. The solution was prepared fresh before use.

Results and Discussion

Perusal of Tables 3 and 4 indicated that LHRH treatment (Group 2) did not modify the expression of oestrus favourably in relation to control (Group 1). Segerson *et al.* (1974) observed that out of 13 progesterone primed ewes treated with luteinizing releasing factor (LRF) only 9 exhibited oestrus, whereas in another group of 10 ewes with similar treatment only 3 exhibited oestrus. Still another report (Rippel *et al.*, 1974) indicates a time lag between hormone administration and behavicural oestrus, which showed that only 75% of the GnRH treated ewes came in cestrus but not until 8 to 10 days after treatment. The findings of Symons *et al.* (1974) further corroborates that GnRH treated ewes did not show behavioural oestrus. In light of these observations, it is, therefore, apparent that the poor response of single injection of LHRH to poor expressivity of behavioural oestrus would be due to its short half life or alternately could result from the lack of optimum concentration of estrogen as was evident from the poor follicular growth.

The mean ovulation time (Table 5) from the onset of oestrus based on 4 hourly oestrus detection and laparotomy was observed to be 28 hr. in the control. In the 3 ewes of group 2 (LHRH and laparotomised) the ovulation time from onset of oestrus was non-significantly different (27.6 hr.) than in case of control group, but when considered from the beginning of the treatment it was less by about 8 hr. (67.29 hr, in the LHRH treated ewes as against 75.0 hr. in the control group).

The overall lambing rate (Table 6) was more or less same (66.7% in the control and 62.2% in LHRH group). However, the duration of oestrus (Table 7) was significantly shorter in the LHRH treated ewes (16 to 24 hr.) as compared to the control group (20 to 40 hr.)

The positive ovulatory response as a result of LHRH treatment was found in 9 out of 11 animals (Table 4). In the progesterone + PMSG treated groups (groups 3 and 4 in the first trial), out of the animals which were laparotomised, ovulation was found to have occurred in

TABLE 5: Probable time of ovulation based on 4 hourly oestrus detection and laparotomy in cycling ewes under hormone treatments.

Description	Treatments			
	Control (Gr. 1)	LHRH (Gr. 2)	Prog. + PMSG (Gr. 3)	Prog. + PMSG (Gr. 4)
No. of ewes laparotomised	1	3	2	2
Ovulation time from onset of oestrus (hr)	28.0	27.6±0.3	47.0±13.0	51.0±4.0
Ovulation time from beginning of treatment (hr)	75.0	67.2±17.4	125.6±14.2	125.5±25.5

TABLE 6. Hormone induced breeding behaviour in cycling ewes.

Groups	No. of ewes	Conceived at				Lambing	
		Ist oestrus		2nd oestrus		No.	%
		No.	%	No.	%		
1	3	2	66.71	—	—	2	66.71
2	3	1	12.50	6	75.00	5	62.25
3	4	—	—	—	—	—	—
4	4	1	25.00	1	25.00	2	50.00
Repeat Expt.							
3	12	1	8.30	—	—	—	—

all ewes. However, in the repeat trial with 10 mg. dose of progesterone+PMSG (Group 3) the ovulation rate was very poor (Table 4). A valid comparison of the treatment can not be made because only one animal in the control group was laparotomised which was found to have ouulated. In the present study in all the ewes in Group 2 the treatment was started on day 16 of the cycle, which was about 2 days ahead of normally expected preovulatory surge of LH as evidenced by the onset of oestrus in the control group. However, ovulation in LHRH treated ewes took place earlier in relation to the time of start of treatment as compared to control. It appears that during follicular phase of cycle, the pituitary sensitivity of exogenous LHRH is increased resulting into an increased release of endogenous LH at that time (Schally *et al.*, 1973). This was fully reflected in response to the given treatment which led to precocious ovulation in 9 out of 11 ewes.

The results obtained in this study showed that although ovulation can be induced by LHRH, the fertility was poor (62.25%). It further indicates that induction of precocious ovulation during follicular phase of the cycle does not provide enough of time for the potentiation of all ovulatory mechanisms during the preovulatory phase resulting in possible immature egg production, poor fertilizing ability and thus low conception rate. Proper onset of oestrus also results in poor mating incidence and consequent low conceptions. This aspect of response may be considered for set-time A.I. programme and if LHRH treatment has to be used for increasing rate of fertility and lambing, the treatment must coincide with normal release of LH.

In groups 3 and 4 (Progesterone+

TABLE 7. Hormone induced oestrus behaviour in cycling ewes.

Group	Interval of onset of oestrus from treatment (hr)	Oestrus duration (hr)	Cycle Length (hr)
1	48.75 ± 1.750^{b}	33.50 ± 1.848^{ab}	17.625 ± 0.378
2	68.33 ± 8.413^{ab}	20.50 ± 1.527^{b}	15.60 ± 1.886
3and 4 (overall combined)	83.66 ± 14.1518^{a}	49.66 ± 3.283^{a}	18.66 ± 0.333

Note: The value with same supesrcripts within each parameter did not differ significantly from each other.

PMSG) aimed at superovulation, behavioural oestrus was much less satisfactory as compared to control (Table 3). Laparotomy observations revealed incidence of silent heat in the treated ewes as evidenced by ovulations (Table 4). Such incidences are not uncommon in progesterone + PMSG therapy. This is supported by the evidence of Gordon (1963) who observed 10% silent heat in progesterone primed ewes receiving 750 I.U. PMSG and by similar other reports (Call *et al.*, 1976). It appears that lack of optimum level of oestrogen in the progesterone + PMSG treated ewes was the reason behind unsatisfactory incidence of overt oestrus expression by the treated ewes, although follicles had developed in them.

The objective of inducing superovulation with different doses of progesterone + PMSG could not be achieved although single ovulations did occur. Because of the failure of progesterone + PMSG treatment to induce superovulations in Group-3, the experiment was repeated during breeding season to ascertain the sensitivity of the gonad to PMSG at high doses. But, surprisingly, ewes recepient of 1000 I.U. and 1500 I.U. of PMSG, too, on observational laparotomy showed similar response as in the previous trial except that only a solitary ewe receiving 1000 I.U. had her both ovaries ovulated while another one with 1500 I.U. PMSG had a single quiet ovulation. The rest four did not ovulate. Even in anoestrus ewes (Laster and Hudson, 1974) and during outside breeding season (Petcu *et al.*, 1977; Sengupta *et al.*, 1976) superovulation has been induced in past with success. In the present experiment the dosage of PMSG used covers a wide range which includes the range of dosage used by above workers who have reported successful superovulation. In the light of this the failure of 'Nali' ewes to superovulate cannot be explained. It seems a peculiar and typical case of breed sensitivity to the exogenous therapy of gonadotrophin which needs further investigation.

Progesterone + PMSG treatment also delayed the ovulation time significantly as compared to control, both from the onset of oestrus as well as when considered from the beginning of treatment (Table 5). The conception rate (Table 6) was also less under progesterone + PMSG treatments. The 10 mg. dose progesterone group failed to conceive completely whereas group receiving 25 mg. dose of progesterone had 50% lambing. Cycle length was also more by a day as compared to control and by about 3 days as compared to LHRH group. On statistical testing these differences were, however, found to be non-significant (Table 7). In conclusion, LHRH and progesterone + PMSG treatments under conditions described in this experiment although increases ovulation rate but is not useful in increasing the lambing rate of sheep of Nali breed.

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Hormonal Control Of Ovulation And Induction Of Superovulation In Barbari Goats Used As Donors In Embryo Transplantation Studies

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ABSTRACT

135 adult female Barbari goats, maintained under normal feeding regime and optimal managerial conditions were administered melengestrol acetate (MGA) in concentrate mixture @ 0.15 mg/animal /day for 16 days. 79 of these goats on the last day of MGA feeding were treated with different amounts of pregnant mare serum gonadotropin (400, 600, 800 and 1000 I.U.) to induce superovulation. The remaining 56 goats were not treated with PMSG and were used as control to study normal ovulation rate.

The oestrus was detected in the morning and evening by parading a vasectomized buck. 46 out of 56 goats treated with MGA alone and 61 out of 79 goats treated with MGA and PMSG exhibited oestrus within 4 days of MGA treatment. The combined treatment with MGA and PMSG seemed to cause no change in oestrus synchronization rate.

The ovulatory response to PMSG treatment varied greatly. The mean ovulation rates and ranges of ovulations in different groups were: control — 1.41 (1-3); 400 i.u. — 2.60 (1-6); 600 i.u. — 3.44 (1-8), 800 i.u. — 5.16 (2-13) & 1000 i.u. — 7.83 (2-12). The PMSG dose as high as 1000 i.u. did not cause any apparent ovarian abnormality.

* * *

Close synchronization of the oestrous cycles of donor and recipient animals and induction of superovulation in the donors are two main necessities and constitute an important facit in the overall embryo transplantation scheme. Various agents have been used for controlling the oestrous cycles and to induce superovulation in farm animals. The use of exogenous gonadotropins (PMSG) in conjunction with progestogen treatment to control ovulation, behavioural oestrus and fertility has been studied in sheep (Hancock and Howell, 1961; Von Rensbrug, 1964; Shelton and Moore, 1971; Laster and Glimp, 1974; Bondioli *et al.*, 1982). Very little work has been done on control of oestrus and induction of superovulation in goats nnder tropical climate. The object of the experiment reported herein was to assess the efficacy of pregnant mare serum gonadotropins (PMSG) in different dose levels in inducing superovulation in Barbari goats pre-treated with melengestrol acetate (MGA), a synthetic progestogen to synchronize oestrus.

Materials and Methods

135 adult female Barbari goats, maintained under normal feeding regime and optimal managerial conditions were

TABLE 1: Occurrence of Oestrus in Barbari goats after MGA and PMSG treatment.

Treatment	No. of animals used	Animals in oestrus (Post-treatment days)									
		Ist day		2nd day		3rd day		4th day		Total animal in oestrus within 4 day	
		No.	%	No.	%	No.	%	No.	%	No.	%
MGA alone	56	Nil	Nill	8	14.29	25	44.64	13	23.21	46	82.14
MGA+ 400 I.U. PMSG	20	Nil	Nil	2	10.00	8	40.00	5	25.00	15	75.00
MGA+ 600 I.U. PMSG	20	Nil	Nil	3	15.00	9	45.00	4	20.00	16	80.00
MGA+ 800 I.U. PMSG	22	Nil	Nil	3	13.63	10	45.45	5	22.72	18	81.81
MGA+1000 I.U. PMSG	17	Nil	Nil	2	11.76	5	29.41	5	29.41	12	70.58
Total = MGA+PMSG	79	Nil	Nil	10	12.66	32	40.51	19	24.05	61	77.22

administered melengestrol acetate (MGA) in concentrate mixture (@ 0.15 mg/animal/ day for 16 days. 79 of these goats on the last day of MGA feeding were treated with different amounts of pregnant mare serum gonadotropins (PMSG) to induce superovulation. Remaining 56 goats were not treated with PMSG and were used as control to study the normal ovulation rate. All experiments were performed within 2 months period (May-June).

PMSG used for superovulation was obtained in the form of freeze—dried powder from M/S Sigma Chemical Company, U.S.A. The powder was reconstituted in sterile normal saline and injected subcutaneously (S/C) on the last day of MGA feeding. The dissolving of the material was completed by vigorous shaking for a few minutes before injection was made. The dose levels and the number of animals used in each group are shown in table 1.

Oestrus was detected in the morning and evening by parading a vasectomized buck. The animals in oestrus were subjected to artificial insemination twice; one at first appearance and second 12 hr. later.

All the nannies were subjected to exploratory laparotomy at 72 hrs. after the onset of oestrus. The ovaries were examined for the number of corpora lutea. The effect of pregnant mare serum gonadotropin was assessed by the rate of ovulations.

Results and Discussion

Control of oestrus (ovulation):

46 out of 56 goats treated with MGA alone and 61 out of 79 goats treated with MGA and PMSG exhibited oestrus within 4 days of MGA treatment (Table 1). The percent exhibition of oestrus in goats given MGA alone was not different from that of goats given MGA+PMSG (82.14 vs 77.22). It is apparent that synchronization of oestrus is not influenced when MGA is used in combination with PMSG.

In this experiment, oestrus synchronization rate of 82% by feeding MGA alone is comparably lower to that obtained by the same worker earlier (Agrawal, 1984) when MGA in the same dose level was fed to goats in October-November months. There seems to be some seasonal effect. Therefore, the effect of micro-environment on heat synchronizing efficiency of MGA needs further investigation on large number of animals in different seasons of the year.

TABLE 2: Ovulation rate in Barbari goats treated with MGA and PMSG.

ATTRIBUTES	DOSE LEVELS OF PMSG				
	MGA alone	MGA+400I.U.	MGA+600 I.U.	MGA+800 I.U.	MGA+1000 I.U.
No. of animals	46	15	16	18	12
Total no. of ovulation points (corpus luteum)	65	39	55	93	94
Ovulation rate (Mean± S.E.)	1.41±0.10	2.61±0.45	3.44±0.52	5.16±0.75	7.83±1.01
Range	1—3	1—6	1—8	2—13	2—12

Induction of superovulation:

Results of ovulation rate in control (MGA alone) and experimental (MGA+PMSG) groups are presented in table 2. The mean ovulation rates and ranges of ovulation were: control —1.4 (1-3); 400 I.U. —2.60 (1-6); 600 I.U. —3.44 (1-8); 800 I.U. —5.16 (2-13) and 1000 I.U. —7.83 (2-12). At least one ovulation was obtained in all the animals.

Superovulatory response was related to the dose levels of PMSG used, though there was high variability in response within the group. Robinson (1951) also observed dose response relationship between the levels of PMSG used and the number of ova shed in sheep.

In the present experiment, the superovulatory response has increased with increasing the dose levels of PMSG. It indicates that exogenous LH or HCG is not required in goats. The presence of large number of fully developed follicles in the ovary in each group indicates that the ovulation rate would have been still more, had some luteinizing hormone been also used on appearance of oestrus.

The PMSG dose as high as 1000 I.U. did not cause any apparent ovarian abnormality. It is concluded from this study that MGA and PMSG can be safely used for control of ovulation (oestrus) and induction of superovulation in Barbari goats in tropical climate.

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Certain Biochemical Studies On Oestrual Vaginal Mucus Of Muzaffarnagri Sheep

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ABSTRACT

The lowest and highest chloride, sodium and potassium content were observed on 8th (589.797±87.823 mg/100 ml) and 36th (714.120±91.910 mg/100 ml) hours, 8th hour (105.805±55.250 mg/100 ml) and 16th day (218.500±44.933 mg/100 ml) and 16th day (31.687±8.055 mg/100 ml) and 36th hour (56.550± 21.450 mg/100 ml) of oestrus cycle respectively. The values of all the three parameters oscillated during the different stages of oestrus cycle. The pH values on 16th day and zero hour of oestrus were close to neutrality, while it tended towards alkalinity at 8 hours of oestrus. The pH values followed an increasing trend from zero to 8 hours and then decreasing trend continued till 16th day of oestrus. The values of ratio between potassium and sodium and total sodium and potassium to chloride and pH value at 8th hour of oestrus showed that pre-oestrual secretions are not quite conductive for sperm survival.

* * *

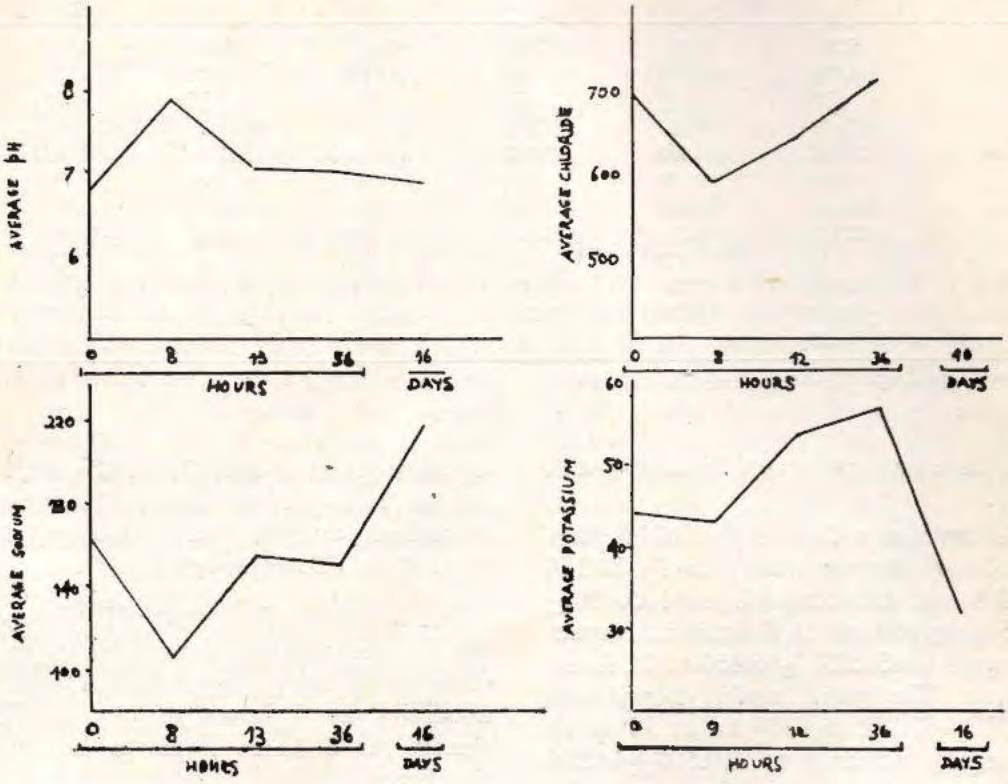
There is general agreement among scientists and sociologists that the world faces today a crisis of rapidly increasing population. There is, therefore, a desperate need for protein to meet the dietary requirements of the people. This means an increasing demand for livestock products, which all nutritionists agree, provide the best answer to this problem. Importance of sheep as source of wool stands today shifted to the "Strategic food-front". Lack of information on different aspects of reproduction of Indian breeds of sheep continues to be limiting factor in the exploitation of the species for maximum production potential. To reduce this gap, biochemical studies of oestrual vaginal mucus were conducted on Muzaffarnagri breed of sheep as no information was available on these parameters. The information on pH, sodium, potassium and chloride contents in vaginal mucus during different phases of oestrus will help in well understanding the different aspects of reproduction in this breed of sheep.

Materials and Methods

As and when an ewe was found in heat, the cervical mucus was collected for the estimation of pH, sodium, potassium and chloride content at 0,8,12 and 36 hours of onset of oestrus and 16th day of the cycle.

Ewe was held and vulval-lips were cleared with dry-cotton and a sterilised speculum was introduced into its vagina by opening vulval lips. Then cervix was located through the speculum and with a long pipette to which a rubber bulb was attached, mucus was sucked and introduced into small sterilized glass vials for further analysis.

FIG. 11- AVERAGE PH, CHLORIDE, SODIUM AND POTASSIUM AT DIFFERENT HOURS AND DAY IN OESTROUS CYCLE IN MUZZAFFARNAGARI EWES.



Immediately after collection, the secretion was subjected to pH measurement by means of BDH pH papers (narrow range). The pH was noted every day in the morning through out the cycle.

Sodium and potassium were estimated by flame photometry method described by Oser (1965) and chloride by Schales and Schales (1941).

The Statistical analysis was carried out according to Snedecor and Cochran (1968).

Results and Discussion

The average chloride content in vaginal mucus at different hours and days in oestrus cycle are presented in table 1 and Fig. 1. The lowest (589.797$\pm$87.823 mg/100 ml) and highest (714.120$\pm$91.910 mg/100 ml) chrolide were recorded at 8 and 36 hours of oestrus respectively.

In oestrus stage, the chrolide was 697.112 mg/100 ml at zero hour, then dropped to 589.797 mg/100 ml at 8 hours and again increased further and reached to its maximum 714.120 mg/100 ml at 36 hours of oestrus. The chloride value again dropped to 606.147 mg/100 ml on 16th day of oestrous cycle. The overall average was 650.135$\pm$73.383 mg/100 ml.

The average sodium in vaginal mucus at different hours and days of oestrus

TABLE 1: Average pH, Chloride, Sodium and Potassium during different phases of oestrus in Muzaffarnagri ewes.

Characters	Hours and Day in Oestrous Cycle					Overall Average ±SE
	0 hour	8 hours	12 hours	36 hours	16th day	
pH	6.79 ±0.0892 (49)	7.90 ±0.4582 (3)	7.05 ±0.950 (2)	7.01 ±0.1224 (4)	6.875 ±0.2975 (4)	7.125 ±0.1992 (61)
Chloride	697.112 ±16.7010 (33)	589.797 ±87.823 (3)	643.501 ±0.000 (1)	714.120 ±91.9103 (3)	606.147 ±111.5701 (4)	650.135 ±73.3827 (44)
Sodium	164.511 ±12.1676 (30)	105.850 ±55.25 (2)	155.250 ±95.45 (2)	150.300 ±61.3218 (3)	128.50 ±44.933 (4)	158.88 ±12.639 (41)
Potassium	43.662 ±3.2865 (30)	42.90 ±3.980 (2)	53.625 ±24.375 (2)	56.550 ±21.45 (2)	31.687 ±8.055 (4)	45.685 ±4.4094 (40)

N.B.: The figures in parentheses are number of ewes.

cycle have been presented in table-1 and fig. 1.

The average sodium at the initial stage (zero-hour) of cycle was 164.511±12.168 mg/100 ml. It decreased to 105.850± 55.250 mg/100 ml at 8 hours and again increased to 155.250±95.450 at 12 hours of cycle. The value again dropped to 150.300±61.322 mg/100 ml at 36 hours and again increased to 218.500±44.933 mg/100 ml on 16th day of oestrus cycle. The lowest sodium was recorded at 8 hours of cycle but the highest sodium content was at 16th day of cycle. The sodium values oscillated during the different stages of oestrus cycle.

The lowest (31.687±8.055 mg/100 ml) and highest (56.550±21.450 mg/100 ml) potassium content in the vaginal mucus was recorded on 16th day and 36 hours of oestrus cycle respectively. During oestrous cycle, the potassium value was 43.662± 3.286 mg/100 ml at zero hour and further decreased to 42.900±3.980 mg/100 ml at 8 hours and again increased to 53.625 ± 24.357 mg/100 ml at 12 hours of cycle. It continued increasing at 36 hours and again dropped to 31.687±8.055 mg/100 ml on 16th day of cycle. The overall potassium average was 45.685±4.409 mg/100 ml (Table 1 and Fig. 1).

It has been noticed that pH values on the 16th day and zero hour of oestrus were close to neutrality while it tended towards alkalinity at 8 hours of oestrus. This change at 8 hours seems to be quite significant.

The corresponding figures for Potassium, Sodium on 16th day showed variation with the different stages of the oestrus. The ratios between potassium and sodium and total sodium and potassium to chloride have been notified in table 2. It could be seen that there is a ratio of 1:7 between potassium to sodium on day 16 and total sodium and potassium to chloride showing a ratio of 1:2.4 with almost neutral pH. This tended to a ratio of 1:4 and 1:3 between sodium to potassium and total sodium and potassium to chloride respectively but the pH still showing a neutrality. At 8 hours of oestrous, the pH was towards

TABLE 2: Ratio of Sodium to Potassium and Chloride to Total Sodium and Potassium at different phases of oestrus in Muzaffarnagri ewes.

Characters	Hours and Day in Oestrous Cycle				
	0 hour	8 hours	12 hours	36 hours	16 day
Sodium/Potassium ratio	3.7678	2.4674	2.8951	2.6578	6.8956
Chloride/Total Sodium and Potassium	3.3487	3.965	3.0808	3.4524	2.4228

alkalinity but the ratio of the sodium to potassium and ratio between total sodium and potassium to chloride attained a ratio of 1:3 and 1:4 respectively.

This shows that pre-oestrual secretions are not quite conducive for sperm survival and probably similar changes may be occuring even in the uterine secretions as the sperms are directly deposited in the uterus in natural service in sheep. Hadek (1953) stated that pH of the oviduct undergoes cyclic changes which were determined by microscopical examination of the ovary. In the present study also the pattern of pH curve is similar but the values differed because pH was calculated from vaginal mucus. Honmode and Pachlag (1973) have also shown the patterns of sodium chloride and pH values during different phases of oestrus cycle and ovulation. The present study is also showing the same pattern in Muzaffarnagri breed of sheep with a slight deviation in values but that might be due to breed difference.

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Gross Observations On Caprine Conceptus (Capra hircus L.): II Growth Of The Components

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ABSTRACT

In all 330 gravid genitalia of local does, 255 with single and 75 with twin foetuses, in various stages of gestation, were collected from abattoir for this study.

Daily growth rate of gravid uterus increased from 2.82 to 7.71 g upto fourth (4.1 to 9.0 cm CRL) stage and rapidly from 22.21 g in fifth (9.1 to 14.0 cm CRL) to 46.21 g in seventh (20.1 to 26.0 cm CRL) and reduced subsequeutly to 19.41 g in ninth (32.1 to 39.0 cm CRL) stage.

Foetal daily growth rate was less (0.029 g to 3.62 g) upto fifth stage but later increased from 9.448 g in sixth to 62.311 g in ninth stage. The daily growth rate of the foetal membranes increased from 0.582 g in second (0.51 to 2.0 cm CRL) to 7.594 g in sixth and reduced thereafter to 1.165 g in seventh stage with a subsequent increase. The daily growth rate of total fluid increased from 1.206 g in first to 11.957 g in sixth stage, thereafter a reduction and fluctuating trend was observed. The growth rate of conceptus increased from 1.82 g in second to 71.57 g in ninth stage.

The polynomial regression curves were drawn with the prediction equations for various parameters of conceptus in early, mid and advanced stage of single and twin pregnancy.

* * *

Materials and Methods

In all 330 gravid caprine genitalia, 255 with single and 75 with twin foetuses in various stages of gestation, were collected from Nagpur Municipal abattoir, for this study. State of gestation was assessed as per Kadu and Kaikini (1984). The average Daily Growth Rate $(W_2 - W_1)$ $(T_2 - T_1)$ and Relative Percentage Growth Rates $(W_2 - W_1) \times 100/W_2$ were calculated for these stages, where W_2 and W_1 are weights at T_2 and T_1 days respectively.

The data were divided into three ranges of C.R. Length of foetus representing the early, mid and late stages of gestation.

The data were analysed by least square regression technique with computer programme with the model.

$$Y = a + bX + cX^2 + dX^3 + eX^4 + ..$$

The polynomial order was obtained to get satisfactory coefficient of determination (R^2-value).

Results and Discussion

Growth rate (Table-1):

The Daily Growth Rate (DGR) of the foetus in single pregnancy was 0.029 g in first stage and increased upto 24.825 g at the end of seventh stage with a slight decrease in eighth and increased to 62.31 g in ninth stage. The Relative Percentage Growth Rate (RPGR) increased from 69.72 percent in first to 81·12

TABLE 1: Average Daily and Relative Percentage Growth Rate of the Components of Conceptus at Various Stages of Gestation in Single and Twin Pregnancy of Goats

Stage	Weight of gravid uterus		Weight of foetus		Weight of foetal memberanes		Weight of total fluid	
	D.G.R. (g)	R.P.G.R.	D.G.R. (g)	R.P.G.R.	D.G.R. (g)	R.P.G.R.	D.G.R. (g)	R.P.G.R.
I	—	—	—	—	—	—	—	—
II	2.82	45.43	0.029	69.72	0.582	91.41	1.206	81.27
III	6.80	56.77	0.151	75.92	0.051	70.34	2.726	64.74
IV	7.71	39.28	0.645	76.38	1.642	43.34	3.748	47.09
V	22.21	53.01	3.626	81.12	3.374	47.11	10.068	5 .31
VI	40.41	49.09	9.448	67.62	7.594	51.46	11.957	39.07
VII	46.21	35.96	24.825	64.07	1.165	7.32	3.707	10.80
VIII	18.74	12.68	26.103	29.36	1.506	8.64	5.981	14.84
IX	19.41	38.23	62.311	53.18	6.940	24.48	2.319	5.44
III	6.03	44.60	0.102	63.22	1.364	58.88	2.010	38.59
IV	21.09	60.88	8.627	79.94	2.98	56.29	11.087	68.56
V	42.06	53.81	3.015	79.98	9.535	64.28	17.013	49.73
VI	51.68	39.80	8.075	67.21	3.467	18.90	24.037	41.44
VII	55.14	42.28	25.432	6 .92	14.023	43.39	10.066	15.34

D.G.R. Daily Growth Rate, R.P.G.R. Relative Percentage Growth Rate.

percent in fifth stage, later on decreased from 67.62 percent in sixth to 53.18 per cent in ninth stage. Similar pattern of growth was observed in twins also.

Thus a shift in the growth patterns was noticed during (75 to 90 days) fifth and sixth stage with a slight change again in late gestation. Similar shift was noticed at these stages by Rattray *et al.* (1974) and was considered normal. It was concluded that foetal growth would not follow a first order relationship. Therefore polynomial regression curves of best fit were obtained. Dun (1955) presented similar growth curves with 90 per cent accuracy.

The weight of the foetus showed 4,3 and 3 degree polynomials for best fit in early, mid and late stages of gestation with 97, 98 and 89 per cent R^2 values, respectively.

I. The resultant polynomials for foetal growth rate for early, mid and late stages were as under

i. $Y = -5.3868 + 11.8671x - 8.6328\,x^2 + 2.7373\,x^3 - 0.02915\,x^4$

ii. $Y = +50.5624 - 17.3359\,x + 1.8513\,x^2 - 0.01883\,x^3$

iii. $Y = -24.03.99 + 364.499\,x - 16.5781\,x^2 + 0.2729\,x^3$

Although a continuous increase in the growth was observed in early, mid and late gestation, boost in growth rate of foetus was observed at 3.1 CRL in early, 15.1 CRL in mid and 30.0 CRL in late stage, corresponding to about 40, 75 and 125 days of gestation in single pregnancy. These obvious points of inflexion and slowing of growth rate was similar to that noted by Cloette (1939) and Rattray *et al.* (1974) inewes. In twin foetuses also similar trend of growth rate was observed.

Foetal membranes:

The DGR of the foetal membranes increased from 0.582 g in second to 7.594 g in sixth stage of single pregnancy. Thereafter, it reduced to 1.165 g and 1.506 in seventh and eighth stage respectively and again increased to 6.940 g in ninth stage. The RPGR was 91.41 in second, reduced to 7.32 in seventh and then increased to 8.64 and 28.48 per cent in eighth and ninth stage respectively. Similar trend was observed in twin pregnancy also.

The foetal membranes showed more rapid growth in earlier stages as compared to that of foetus to meet its impending biological requirements. In later stage, however, the trend of growth rate was just the reverse. These finding confirmed the earlier reports in sheep by cloette (1939), Wallace (1948) and Rattray *et al.* (1974).

The average weight of the foetal membranes during early, mid and late single pregnancy could best be described by 3, 3 and 3 degree of polynomials with R^2 values of 55.8, 61.3 and 38.9 per cent respectively.

II. The resultant polynomials of the three stages for growth rate of foetal membranes were as under in single pregnancy.

i. $Y = 14.1826 - 25.2246\ x + 18.2499\ x^2 - 2.6127\ x^3$

ii. $Y = 130.3203 - 31.0156\ x + 3.4765\ x^2 - 0.0801\ x^3$

iii. $Y = 2164.99 + 257.99\ x - 9.3515\ x^2 + 0.1152\ x^3$

The growth curve of foetal membrances could be best described by 3 degree polynomials in all the three stages with about 60 percent R^2 value which was in accordance with studies reported by Cloette (1939), Wallace (1948) and Alexander (1964).

Total foetal fluids:

Total foetal fluids improved distinctly from 1.206 g to 11.95 g per day from second to sixth stage & declining subsequently. Although, the trend was similar in twin pregnancy, the daily increase was about twice that of singles during above period. Robinson *et al.* (1977) showed similar decrease in ewes. The trend of increase in RPGR however, was similar in single and twins. Growth curves for total foetal fluid were obtained with 2, 4 and 7 degree of polynomial regressions with 54, 81 and 31 percent R^2 values indicating much variations in the individual observations.

III. The resultant polynomials of average volume of total fluid in early, mid and late stages were as under:

i. $Y = 10.7094 + 21.1119\ x + 0.6338\ x^2$

ii. $Y = -341.49 + 188.625\ x - 30.4218\ x^2 + 2.29\ x^3 - 0.0578\ x^4$

iii. $Y = -1175.99 - 39.99\ x + 19.249\ x^2 - 0.8320\ x^3 + 0.0168\ x^4 - 0.0008\ x^5 - 0.0000309\ x^6 - 0.00000034\ x^7$

Similar high variability was also reported by Lyngset (1971) and Bongso *et al.* (1979) in twin pregnancy.

Although present study deals with the highly heterogeneous polutation, the trend of observations is sufficiently marked and could thus be utilized for a better understanding and improving the inherent reproductive potential in goats.

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Studies On Reproduction In Sheep. 6. Effect Of Gestation Length And Dam's Weight At Service And Lambing On Pre-weaning Body Weight In Exotic Fine Wool Breeds

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ABSTRACT

Lambing of 249 Russian Merino and Rambouillet sheep were studied under semi-arid conditions. Relationship between preweaning body weights of lambs and dam's weight at different stages of reproduction and gestation length were studied. Significant ($P<0.05$) differences were observed between breeds in dam's weight at lambing, gestation length and pre-weaning body weights. Rambouillet ewes were lighter than Russian Merino ewes at lambing and they carried single lambs for a longer period, whereas reverse was true in case of twin births. Preweaning weights were heavier in case of Russian Merinos.

* * *

The factors viz dam's., age, dam's weight, type of birth and type of rearing which affect milk yield, have significant effect of pre-weaning growth of lambs. In this paper, the effect of gestation length, body weights of dam's at service and lambing, and sex on pre-weaning growth of lambs in case of exotic fine wool breeds were examined, for securing improvement in reproductive efficiency under intensive farming conditions.

Materials and Methods

Observations were recorded on Merino ewes imported from Russia in October, 1971 and Rambouillet ewes imported from Texas (U.S.A.) in December, 1964. The data pertaining to lambings, of Russian merino and Rambouillet, formed the basis of this study. Russian Merino were 2-3 years old and Rambouillet 2-8 years old at the start of this study. Management of the ewes and lambs has been described in detail somewhere else (Kaushish and Sahni, 1977).

The ewes detected in heat in the morning were bred through artificial insemination on the same day and those detected in the evening were bred in the morning on the next day. Body weights of the ewes were recorded before breeding and at lambing and of lambs within 12h of birth and at the age of 1, 2 and 3 months of age. The sex of the lamb and the type of birth were also recorded at birth.

Most of the ewes were multiparous. The gestations terminating in the birth of normal lamb were included in this study. The duration of pregnancy was calculated from the day of effective service to the day of parturition. The data was analysed by weighted analysis as suggested by Snedecor and Cochran (1967)

Results and Discussion

The average values for gestation length, weight at service and lambing and pre-

TABLE 1: Averages (Mean±S.E.) of gestation length, weight at service and lambing of ewes and body weight of single and twin births.

Attributes	Single births				Twin births			
	Rambouillet		Russian Merino		Rambouillet		Russian Merino	
	Male	Female	Male	Female	Male	Female	Male	Female
No. of observations	35	23	49	53	20	15	30	24
Gestation Length (days)	149.30 ±0.38	149·60 ±0.50	148.80 ±0.34	148.80 ±0.32	148.10 ±0.46	148.50 ±0.52	149.00 ±0.40	148.80 ±0.44
Weight at service (Kg)	43.27 ±1.018	48.81 ±2.523	47.31 ±0.763	46·85 ±0.798	45.63 ±1.175	47.17 ±1.420	50.74 ±0.398	52.70 ±0.846
Weight at lambing (Kg)	44.37 ±1.226	42.50 ±1.809	47.21 ±0.941	45.64 ±0.896	45.77 +1.268	46.23 ±1.745	48.89 ±0.921	52.50 ±1.226
Birth weight (Kg)	3.77 ±0.119	3.33 ±0.148	4.09 ±0.080	3.33 ±0.148	2.76 +0.135	2.62 ±0.130	2.93 ±0.091	3.07 ±0.123
One month weight (Kg)	8.43 ±0.302	7.25 ±0.376	10.41 ±0.231	9.20 ±0.259	6.31 +0.351	6.68 ±0.433	7.80 ±0.295	7.82 ±0.328
Two month weight (Kg)	11.97 ±0.427	10.68 ±0.546	14.69 ±0.353	13.32 ±0.398	9.68 +0.640	9.86 ±0.682	11.59 ±0.505	11.25 ±0.521
Three month weight (Kg)	14.77 ±0.594	12.91 ±0.892	17.12 ±0.412	16.50 ±0.452	12.03 +0.791	11.75 ±0.802	14.86 ±0.649	14.11 ±0.634

weaning body weights of lambs in Russian Merino and Rambouillet breeds are presented in Table-1.

Effect of gestation length: The average gestation period ranged from 148.99 days in Rambouillet to 148.83 days in Russian Merino ewes. There were significant differences in gestation length between breeds in case of single births only. It was shorter than that reported by Terril and Hazel (1947) in case of Targhees and Corriedale, but was longer than that reported by Ostgard (1957) in case of Spaelson, Cheviots and Dalas.

Sex of the lamb born did not affect the gestation length which agreed with those reported by Kelley (1943) and Van Niekerk and Mulder (1965). The relationship between gestation length and pre-weaning body weights in case of Rambouillet and Russian Merino breeds was not statistically significant. This does not agree with the findings of Kaushish and Arora (1974), who observed significant correlation between gestation length and birth weight. In contrast, Sharma *et al.* (1978) reported that the effect of gestation length on body weights was not significant for all ages, except at 13th week. This difference could be due to the difference of breed, because the above workers observed this relationship in case of coarse wool breeds of sheep native to subtropics.

Effect of weight at service: On an average Russian Merino ewes were heavier (48.640 Kg) than Rambouillet ewes (45.77 kg) at the time of breeding. The difference due to breed was observed to be significant ($P<0.05$). The ewes which were heavier in weight at breeding, gave birth to heavier lambs indicates a direct relationship between these parameters. Weight at service was positively correlated with pre-weaning body weights of lambs in case of Russian Merino and Rambouillet sheep. However, it was not so in case of twin birth in Russian Merino and Rambouillet ewes except at birth and one month of age in Rambouillet

breed. This agrees with the findings of Bhasin and Desai (1967) and Kaushish (1971) in case of native and Terril and Hazel (1947) and Coop and Hayman (1962) in case of exotic breeds. These workers reported significance of body weight of dam at service on birth weight of lambs. Sharma *et al.* (1978) reported that weight of dam at service was not only important from birth weight of lambs but also for subsequent body weights. Our results are in agreement with the findings of Sharma *et al.* (1978).

Effect of weight at lambing: The Russian Merino sheep were heavier than Rambouillet sheep at the time of lambing. The average weight in respective breeds at lambing were 47.81 and 44.51 Kg. The difference between breeds was significant. The weight at lambing was positively correlated ($P<0.01$) with pre-weaning body weights in case of Russian Merino as well as Rambouillet breed except in case of twin births in the former breed. Dam's weight at lambing significantly influenced ($P<0.01$) the body weights at all the ages. This is in agreement with the findings of Nivsarkar *et al.* (1984)

Effect of sex: The effect of sex of lamb was significant ($P<0.01$) in case of single births only. Male lambs on an average were heavier than the female lambs in both the breeds. Sharma *et al.* (1978) did not find significant differences between sexes upto weaning, however Singh & Prashad (1962) reported difference in birth weight in both sexes. Further Dass and Acharya (1970) and Chopra and Acharya (1971) reported that sex of lamb significantly affected birth weight and body weights at different ages.

From these, it can be concluded that higher the weight of sheep at service and lambing and longer the gestation period, heavier will be lambs up to pre-weaning age.

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A Study On Post-Partum Oestrus Interval In Muzaffarnagri Sheep

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ABSTRACT

A study on post-partum oestrus interval was carried out to determine the effects of season and sex of the lamb on this trait in Muzaffarnagri breed of sheep. The post-partum oestrus interval averaged 137.4 $\pm$ 4.82, 168.1 $\pm$ 23.63 and 172.8 $\pm$ 13.22 days following single normal lambing, twin lambing and abortions based on 309, 11 and 57 observations, respectively.

For single normal lambing, the sex of the lamb did not have significant effect on post-partum oestrus interval. Also there was no significant difference in this trait due to seasons. The frequency distribution revealed that 19.1% of ewes came in heat within 60 days after lambing with an average of 35.5$\pm$1.94 days.

The average post-partum oestrus interval following twin lambing and after abortions was abnormally long. The effect due to seasons was significant ($P<0.05$) on this trait. Maximum number of ewes (28.1%) came in heat in the range of 151-180 days after abortions with an average of 167.4$\pm$1.90 days. It was observed that no ewes aborted during the hot-dry season. Similarly only one case of twin normal lambing was noticed during this season in Muzaffarnagri sheep.

* * *

In this paper, the period lapsed between lambing and the occurrence of first oestrus after lambing, is reported in Muzaffarnagri sheep following single normal lambing, twin lambing and abortions. The effects of season and sex of the lamb are also investigated on this trait.

Materials and Methods

A total of 377 ewes comprising of 309 ewes of single normal birth, 11 ewes of twin normal birth and 57 ewes with abortion cases were used to study the post-partum oestrus interval in Muzaffarnagri sheep maintained at the IVRI, Izatnagar farm under the All India Coordinated Research Project on Sheep for Mutton.

Ewes after lambing were housed in a separate shed which had also a paddock. All the ewes were fed with concentrate and green fodder in the stall and were suckled by their lambs.

Oestrus was detected in these ewes by a vasectomised teaser ram. Heat detection was made from the second day onward of lambing till the ewes evinced first oestrus. The teaser ram was run in the shed twice daily for one hour both in the morning and evening and the ewes coming in heat were identified. During heat detection, young lambs were removed from their dams and kept separately. The data on lambing for each individual ewe were used for calculating the period between lambing and the occurrence of first

TABLE 1. Averages, Standard errors and Coefficients of variation alongwith frequency distribution of post-partum oestrus interval (days) in Muzaffarnagri sheep following single normal lambing

Interval (days)	No. of ewes	Percentage of ewes	Mean	SE	CV%
≤60	59	19.1	35.5	1.94	5.46
61-90	51	16.5	77.6	1.18	1.51
91-120	38	12.3	103.9	1.27	1.22
121-150	38	12.3	137.1	1.28	0.93
151-180	40	12.9	163.8	1.38	0.84
181-210	28	9.1	196.0	1.60	0.81
211-240	22	7.1	229.5	2.14	0.93
241-270	7	2.3	254.1	2.70	1.06
271-300	12	3.9	285.2	2.22	0.77
301-330	5	1.6	320.2	2.58	0.80
331-360	3	1.0	344.0	6.56	1.90
>360	6	1.9	386.0	12.16	3.15
Total	309	100.0	137.4	4.81	3.50

oestrus after lambing. The interval was called as the post-partum oestrus interval (days) which is the ultimate trait studied and reported in this paper.

To examine the effect of seaons on this trait, the year was divided into 4 seasons based on temperature and humidity as follows:

1. Cold season: Second half of November to February.
2. Temperate: season — Second half of September to first half of November and March to first half of April.
3. Hot-dry: season — Second half of April to first half of June.
4. Hot-humid: season — Second half of June to first half of September.

TABLE 2. Averages, Standard errors and Coefficients of variation alongwith frequency distribution of post-partum oestrus interval (days) after abortion in Muzaffarnagri sheep

Interval (days)	No. of ewes	Percentage of ewes	Mean	SE	CV%
≤60	8	14.0	35.5	6.20	17.46
61-90	3	5.3	77.0	1.00	1.29
91-120	4	7.0	101.0	3.10	3.06
121-150	4	7.0	135.0	4.77	3.53
151-180	16	28.1	167.4	1.90	1.13
181-210	10	17.5	194.8	2.95	1.51
211-240	1	1.8	240.0	—	—
241-270	4	7.0	249.8	3.11	1.24
271-300	3	5.3	272.7	1.53	0.56
>300	4	7.0	426.7	47.06	11.02
Total	57	100.0	172.8	13.22	7.65

The analyses of the data were carried nut as per Snedecor and Cochran (1968).

Results and Discussion

The averages, standard errors and coefficients of variation for post-partum oestrus in Muzaffarnagri ewes alongwith frequency distribution following single normal lambing are given in Table 1 and after abortions in Table 2. The shortest post-partum oestrus interval after single, twin normal births and abortions was 2, 8 and 4 days and the longest in the same order was of 443, 253 and 519 days. The observed ranged was divided into various classes of 30 days duration except the first which was 60 days.

The frequency distribution revealed that maximum number of ewes (19.1%) came in heat with in 60 days after lambing with an average of 35.5 ± 1.94 days. Similarly 16.5% ewes depicted post-partum oestrus after an interval of 77.6 ± 1.18 days with a range of 61-90 days. The percentage of animals went on decreasing as the post-partum oestrus interval increased till 240 days(Table 1). This was in the case of ewes following single normal lambing.

Table 2 revealed that the maximum number of ewes (28.1%) came in heat in the range of 151-180 days after abortion with an average of 167.4 ± 1.90 days. Similarly 17.5% ewes depicted post-partum oestrus after an average interval of 194.8 ± 2.95 days with a range of 181-210 days. Fourteen percent ewes came in heat within 60 days after abortion with an average of 35.5 ± 6.20 days.

Sahni and Roy (1967) in Bikaneri sheep reported average post-partum heat interval as 41.6 days with a range of 17-93 days. They found that nearly 70% of the ewes came in heat between 30-50 days post-partum which is not in agreement with the present findings. Taparia (1972) studied post-partum oestrus in Sonadi sheep which varied from 28-93 days with a mean of 62 days. Majority of ewes showed heat between 40 and 80 days after lambing. It was noted that post-partum heat interval in Muzaffarnagri sheep is much longer than in other Indian breeds of sheep as reported by Sahni and Roy (1967) and Taparia (1972). The probable reason for such a variation might be due to breed or management differences in the case of single normal lambing.

Based on 166 observations dne to male lambing, the post-partum oestrus interval averaged 137.0 ± 7.06 days and that due to female lambing averaged 137.8 ± 6.49 days in 143 ewes. It was observed that there was no significant difference in the post-partum oestrus interval due to sex of the lamb in Muzaffarnagri sheep.

Table 3 gives the averages and standard errors of the post-partum oestrus interval after single normal birth, twin normal birth and abortions in different seasons of the year.

It can be seen from Table 3 that ewes following single normal lambing came into heat little earlier in Hot-dry and Hot-humid seasons than in other two seasons. However, there was no significant difference in this trait due to seasons (Table 4). This finding indicated that the environmental conditions including managemental fluctuations and quality of feed and fodder did not have variable effects on post-partum oestrus interval and the ewes did not behave differently in different seasons of the year so far as post-partum oestrus interval after single normal birth in Muzaffarnagri sheep is concerned.

The post-partum heat interval followed by twin lambing averaged 168.1 ± 23.63

TABLE 3: Averages and Standard errors of post-partum oestrus interval (days) after single normal birth, twin normal birth and abortion in different seasons

Seasons	Single normal birth			Twin normal birth			Abortion		
	N	Mean	SE	N	Mean	SE	N	Mean	SE
Cold	96	146.9	7.51	2	181.5	23.56	15	129.7	13.73
Temperate	92	140.4	8.49	5	150.2	24.23	36	200.3	17.92
Hot-dry	43	122.6	13.70	1	8.0	—	0	—	—
Hot-humid	78	130.2	11.06	3	245.0	8.51	6	115.2	35.55
Overall	309	137.4	4.82	11	168.1	23.63	57	172.8	13.22

N = No. of observations.

TABLE 4: Analysis of variance showing the effect of season on post-partum oestrus interval

Source	Single normal birth		Twin normal birth		Abortion	
	df	MS	df	MS	df	MS
Season	3	7663	3	16008*	2	37521*
Error	305	7188	7	1880	54	8924

* Significant at 5% probability level.

days which was abnormally long probably due to small sample size and more time required for involution of uterus over stretched due to twinning. Although the effect associated with seasons was significant ($P<0.05$), nothing conclusive could be said for want of sufficient data.

Fifty seven observations were available on post-partum oestrus interval after abortions. This averaged 172.8 ± 13.22 days which was also abnormally long due to abnormal births i.e. abortion cases. Here also the effect due to seasons on this trait was significant ($P<0.05$). The average post-partum oestrus interval during temperate season was found to be significantly longer as compared to those in cold and hot-humid seasons. The interesting observation was that no ewes aborted during the hot-dry season. This may be due to less favourable climate for infectious agents causing abortions. Only one case of twin normal lambing was observed during this season.

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Studies On Certain Characters Of Spermatozoa Obtained From Epididymis And Vas Deferens Of Black Bengal Goat: 2. Mensuration, Succinic Dehydrogenase And Melanizing Activities

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ABSTRACT

Luminal contents of three different regions of epididymis viz., caput, corpus and cauda, and vas deferens were collected from 45 Black Bengal bucks by 'open method' of castration. Mensuration characters, succinic dehydrogenase and melanizing aetivities of midpieces of spermatozoa and their interrelationship were determined. The length of midpieces of spermatozoa were gradually decreased while both the succinic dehydrogenase and melanizing activities were increased progressively during their transit from caput to vas deferens. All the differences in the value of succinic dehydrogenase activities except in between cauda and vas deferens were significant. It may be concluded that the length and area of midpieces of spermatozoa were inversely related with the succinic dehydrogenase and melanizing activities.

* * *

In our previous study (Bhattacharjee *et al.*, 1985), we reported certain morphological characters like livability, cytoplasmic droplets and resistance to cold shock of spermatozoa from epididymis and vas deferens of Black Bengal bucks and their interrelationship. It was observed there that the maturation of spermatozoa was nearly completed within cauda epididymis and vas deferens. Maturation process involves certain metabolic activities which were reported to be confined in the midpiece of spermatozoa (Ayyagari and Mukherjee, 1970). Therefore, we nave studied some mensuration characters of midpiece of spermatozoa obtained from different parts of epididymis and vas deferens of Black Bengal bucks alongwith certain enzyme activities e.g, succinic dehydrogenase and melanizing activities and tried to find out their interrelationship.

Materials and Methods

Adult healthy Black Bengal bucks maintained at the farm of Bidhan Chandra Krishi Viswavidyalaya were considered. Each buck was castrated by 'open method' (O'Conner, 1980). Castration was done on every alternate day and the process involved 45 bucks. The testes (right and left) of each were then placed into a beaker containing normal saline solution (NSS) at room temperature. The tunica albuginea was carefully removed from each testis and ligatures were placed at proximal portions of ductus deferens, ampulla cauda and distal to caput, ductuli efferntia. Epididymis along with vas deferens were dissected out from each testis and kept immersed

TABLE 1: Mensuration characteristics of spermatozoa midpiece obtained from different regions of epididymis and vas deferens (Mean values of 360 observations with SE)

	Midpiece		
	Length (μ)	Breadth (μ)	Area (μ^2)
Epididymis:			
a) Caput	9.61a	0.53c	5.09g
	±0.06	±0.006	±0.05
b) Corpus	9.54a	0.52c	5.00g
	±0.06	±0.006	±0.05
c) Cauda	9.34b	0.53c	4.98g
	±0.05	±0.006	±0.06
Vas deferens	9.29b	0.52c	4.85g
	±0.06	±0.007	±0.06

Values having dissimilar scripts vary significntly ($P < 0.01$) from each other within the same column.

in NSS. The vas deferens were carefully then separated out and kept in watch-glass. The epididymis was carefully segmented into caput, corpus and cauda and kept separately on three watch-glasses. They were incised longitudinally and flushed with 2 ml NSS with the help of micro-pipette so as to obtain luminal contents of above four parts separately and vortexed for 30 sec. Two permanent slides were made from each luminal content and in the process 360 slides were made. They were then coded and four spermatozoa in each slide were examined in randomized order for determining the midpiece length, breadth and area.

For determining dehydrogenase activity, one drop of luminal content of caput, corpus, cauda and vas deferens were incubated separately in four watchglasses at 37°C for 1 hr alongwith one drop of 0.1M sodium succinate and incubating media (10 mg of nitro BT in 10 ml of 0.1M phosphate buffer at pH 7.00). Two smears were prepared from each sample, dried and fixed in formo-saline. The slides were then washed for 3 min in running tap water and stained with 1% solution of eosin-Y. In the process 360 slides were made, coded and examined in randomised order under oil immersion lens of microscope. The dehydrogenase activity of spermatozoa was then expressed and classified on the basis of colour intensity, size and density of granules in mitochondria (Hrudha, 1965). The results of mensuration character of midpiece and succinic dehydrogenase activity were analysed statistically (Snedecor, 1959).

For determining melanizing activity, one drop of semen from each of the caput, corpus, cauda and vas deferens was diluted with NSS in the proportion of 1:100. One ml of diluted semen was then mixed with equal volume of 0·1% DOPA in NSS and incubated for 6 hr at 25°C. The enzyme activity was then expressed following the method of Beatty (1956) based on the intensity of colour.

Results and Discussion

The length, breadth and area of the midpiece of the spermatozoa obtained from different regions of epididymis and vas deferens are presented in Table 1. It has been observed that

TABLE 2: Succinic dehydrogenase activity of spermatozoa obtained from different regions of epididymis and vas deferens (Mean values of 360 observations with SE)

	Scoring scale based on the intensity of colour development in mitochondrial sheath			
	Highly stained with large granules (+++)	Medium stained with loosely packed granules (++)	Weakly stained (+)	Diffusely stained (—)
Epididymis:				
a) Caput	34.11a ±0.44	27.29d ±0.48	34.45g ±0.36	22.42j ±0.34
b) Corpus	37.94b ±0.57	34.33e ±0.37	26.60h ±0.45	17.89k ±0.37
c) Cauda	47.28c ±0.38	31.61b ±0.35	19.84i ±0.49	14.14l ±0.40
Vas deferens	47.97c ±0.38	32.21f ±0.41	19.01i ±0.43	12.85m ±0.37

Values having dissimilar superscripts vary significantly ($P < 0.01$) from each other.

the length and area of the midpieces of spermatozoa decreased gradually while traversing through the epididymis and the differences in the value of different regions were not statistically significant except in between caput & cauda, and corpus & vas deferens in respect of length. The observations corroborated with the findings of Mukherjee and Bhattacherjee (1949), Osman (1973), Glover (1974) and Laufer *et al.* (1979), while disagreed with the findings of Jindal and Panda (1980), and Mazumdar *et al.* (1981) where they reported that the breadth of the caudal spermatozoa were greater than that of caput epididymis in goat. Likewise, the presence of cytoplasmic droplets and resistance to cold shock spermatozoa were decreased progressively during their transit from caput of epididymis to vas deferens (Bhattacharjee *et al*, 1985) in goat. The epididymal secretion possessed a higher osomotic pressnre compared to blcod and this might have caused dehydration of the spermatozoa (Lindahal and Khilstrom, 1952 and Salisbury *et al.*, 1978) leading to the decrease in shape and size of the midpieces of spermatozoa of the present findings. Therefore, it might be assumed that the decrease of midpiece length and area are an integral parts during the prccess of maturation of spermatozoa.

The succinic dehydrogenase activities of the midpieces increased considerably while traversing through the different regions of epididymis and vas deferens (Table 2). All the differences in the values of enzyme activities except in between cauda and vasdeferens were statistically significant ($P<0.01$). The dehydrogenase activity is intimately associated with the metabolism of spermatozoa. After spermatcgenesis, testicular spermatozoa traverses through the highly convoluted epididymis and vas deferens, and undergoes maturation process in Black Bengal Goat (Bhattacharjee *et al.*, 1985). The maturation involves several functional changes like develcpment of sustained motility, fertility and gradual increase in metabolism. This might have been the possible reason of increased dehydrogenase activity of midpieces of spermatozoa

TABLE 3: Melanizing activity of spermatozoa obtained from different regions of epididymis and vas deferens of Black Bengal buck (45 observations)

	Scoring scale based on colour development					
	No colour '0'	Definite trace requiring close inspection (+)	Trace, at once evident +	Brown ++	Nearly black +++	Black ++++
Epididymis:						
a) Caput	16	11	18	—	—	—
b) Corpus	2	—	27	16	—	—
c) Cauda	—	—	—	8	20	17
Vas deferens	—	—	—	—	30	15

obtained from cauda and corpus epididymis. It was reported earlier that the maturation of spermatozoa was almost complete in the cauda (Bhattacharjee *et al.*, 1985) and probably this might have been the possible reason for not getting any significant differences in the dehydrogenase activity of the midpieces of spermatozoa collected from cauda of epididymis and vas deferens. This also further showed that the maturation of spermatozoa demands greater need of energy through oxidative metabolic pathway of carbohydrate metabolism.

The melanizing activities of spermatozoa obtained from different regions of epididymis and vas deferens are presented in Table 3. The enyzme activities increased progressively as it passes through different regions of epididymis and the maximum activity was observed in the spermatozoa of vasdeferens. This showed that the maturation, metabolism and melanizing activity are directly related with livability of spermatozoa. Therefore, melanin formation is an integral part of metabolism and associated with the process of maturation. But, Mukherjee (1969), Pant and Mukerjee (1971), and Misra and Mukherjee(1979) reported that melanizing activity is inversely related with succinic dehydrogenase and livability of spermatozoa of ejaculated semen. Ejaculated semen contains seminal plasma and probably the action of seminal plasma is responsible for the differences in the melanizing activity of spermatozoa obtained from ejaculation, and epididymis and vas deferens. Therefore, it may be conclued that the progressive decrease of midpiece length and area of sperm at ozoa alongwith the concomitant increase of succinic dehydrogenase and melanizing activity indicated that they are inversely related.

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SHORT COMMUNICATIONS

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Metronidazole (Flagyl) In The Treatment Of Anaerobic Genital Infections In Buffaloes

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ABSTRACT

A total of 123 Surti buffaloes which were previously refractory to the usual antibiotic treatment for reproductive disorders (Endometritis, 55 and repeat breeding buffaloes, 68) were treated with 0·5 % metronidazole (Flagyl, M. & B. Pvt. Ltd., Bombay) intra-uterine for 3 days regularly and as post-insemination treatment at the Veterinary College A.I. Clinic, Anand. Out of 61 (50%) buffaloes that could be followed, 20 (32.79%) became pregnant with an average interval of 52·5 days post-treatment and required 1·95 inseminations/ conception. Metronidazole was found to be effective in 40% buffaloes with endometritis and in 29·27% repeating buffaloes with a mean interval of fertile oestrus as 63·75 and 45·00 day, respectively.

* * *

Bovine infectious infertility is of great concern to the Indian dairy economy. A lot many research has been conducted on isolation of aerobic microflora of normal and diseased genital tract of females and the remedial measures undertaken according to their sensitivity eo various antibiotics (Namboodripad tt *al.*, 1978; Kavani, 1983). But much more work is still required to understand the role of anaerobic infection in the genital tract of female animals. The non-sporing anaerobes particularly *Bacteroides* are responsible for a wide variety of infections especially sepsis of gastrointestinal tract and of the female genital tract in human (Nalini *et el.*, 1978; Singh *et al.*, 1978; Bhargawa *et al.*, 1978 and Joshi, 1978).

However, with the exception of *Fusobacter* and *Actinomyces* the literature on non-sporing anaerobes in animals is sparse but based on experience in man, it is logical to assume that such infections do occur in animals. Some indication that it mignt be so was given by reports on the successful use of metronidazole in a wide range of conditions in small animals that had been previously refractory to other antimicrobial therapy (Carwardine, 1984).

In India, anaerobic bacteria viz., *Peptococci*, *Peptostreptococci*, *Bacteroides* and *Clostridia* have been isolated by Hukeri *et al.* (1982) from the uterus of 33 buffaloes with abortion, post-partum metritis and repeat breeding conditions. They found use of 0·5% metronidazole intra-uterine to be encouraging when used according to anaerobic isolates' sensitivity testing from these cases.

TABLE 1. Response to metronidazole (Flagyl) therapy in buffaloes with anaerobic infections of reproductive disorders.

Sr. No.	Reproductive disorders	No. of cases treated	No. followed	No. pregnant	Conception %	Post treatment fertile oestrus interval (days)	No. of AI/ Conception
1.	Endometritis	55	20	08	40.00	63.75	1.75
2.	Repeat breeding	68	41	12	29.27	45.00	2.08
	Overall	123	61	20	32.79	52.50	1.95

The present day practice of indiscriminate use of higher antibiotics has created a most vital problem of drug resistance in animal treatment and also the flare-up of mycotic and anaerobic infections. Many animals are remaining unproductive with or without visible genital infections and are refractory to usual antibiotic therapy. Therefore, the present study was undertaken to know the efficacy of metronidazole in such buffaloes to rule out the possibility of anaerobic genital infections as a cause of reproductive failure.

Materials and Methods

A total of 123 Surti buffaloes having reproductive disorders (55 with endometritis and 68 repeat breeding) that were brought by the farmers at the Veterinary College A.I. Clinic, Anand for the treatment were included in the study. In most of the endometritis cases, there was a thick purulent foul smelling discharge. All these animals were previously treated with commonly available antibiotics with or without sensitivity testing of their aerobic isolates but were found refractory to in-vivo therapy. Majority of them were also resistant to usual antibiotic sensitivity testing in-vitro. Therefore, assuming the role of anaerobic bacteria in these cases, they were treated with 0·5% metronidazole intra-uterine at a dose rate of 15 ml daily for 3 days and also as post-insemination treatment with single dose half an hour after insemination. Then the nature of discharge and clinical response was judged and recorded after per-rectal palpation 10-15 days later. Animals were inseminated during their subsequent oestrus wherever the oestrual discharge was apparently normal. Pregnancy diagnosis was made 2—2½ months after the last insemination.

Results and Discussion

In all 123 Surti buffaloes with reproductive disorders (55 with endometritis and 68 repeating buffaloes) were treated with 0·5% metronidazole intra-uterine after their being refractory to usual routine antibiotic therapy on the basis of sensitivity testing of few represeutative aerobic isolates in-vitro. However, the isolation, identificatiou and sensitivity of anaerobes to metronidazole could not be ascertained due to want of sophisticated facilities required for this. But on the basis of refractory results of commonly used antibiotics, it was assumed that the anaerobic infections may be responsible in these cases for the infertility and as such metronidazole was used. The details of animals treated, followed and the subsequent results are presented in table 1.

The table shows that in infectious reproductive disorders (endometritis and repeat breeding) anaerobic bacteria can play a significant role in their causation. All these animals were refractory to routine culture and sensitivity tested therapy but metronidazole could help to settle them and resulted in clinical recovery of 40% buffaloes with endometritis and 29·27% repeat breeding cases among followed with a mean fertile oestrus interval of 63·75 and 45·00 days post-treatment and requiring 1.75 and 2.08 inseminations/ conception, respectively.

Amongst followed, those buffaloes which did not conceive had persistent visible genital infection even after treatment with metronidazole. Repeating animals had irregular or regular oestrus aud oestrus cycle. There were alterations in the cervical morphology and its secretion.

Bovine uterus is the most suitable place for growth of anaerobic bacteria which also makes a part of normal flora and thus is a major source of reproductive disorders due to infection especially when they invade adjacent tissues. Such invasion may occur following trauma, inter-current infections, failure of local immune mechanism which normally maintains the status quo or following the indiscriminate use of broad spectrum antibiotics where ecological balance may be tilted in favour of a resistant anaerobe by elimination of sensitive organisms which were formerly the dominent species (Hukeri *et al.*, 1982 and Carwardine, 1984).

Many anaerobes are resistant to the commonly used antibiotics such as penicillin and oxytetracycline. Many produced penicillinase and to make matter worse, some produced an enzyme that inhibited phagocytosis (Ingham *et al.*, 1977), thus reducing the body's defence mechanism and the efficacy of bacteriostatic drugs.

Fortunately, metronidazole which is only drug effective against anaerobic bacteria, is relatively non-toxic, has ability to diffuse through deeper tissues, resistance is virtually non-existent, is compatible with other antimicrobial agents and has been successful in the treatment of wide range of refractory infections; was used in the present study and was found to be encouraging as has been reported by study of Hukeri *et al.* (1982), in the reproductive disorders of buffaloes. However, it would be advantageous if isolation and sensitivity of anaerobes could be determined and accordingly the treatment is carried out for maximum recovery.

Acknowledgement

The authors are grateful to Dr. M.R. Patel, Principal, Gujarat Veterinary College, Anand for providing facilities for the work. Thanks are also due to Mr. B.V. Agnihotri, Manager — Animal Health, May & Baker (India) Ltd., Bombay for supply of free samples of FLAGYL for the trial.

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Occurrence Of First Post-Partum Oestrus Under Field Condition In Surti Buffaloes.

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ABSTRACT

Occurrence of first post-partum oestrus (FPPO) was studied in 755 Surti buffaloes, brought at the Gujarat Veterinary College A.I. Clinic, Anand for a period of one year (1984-85). The study revealed that 67·09% FPPO's were exhibited during high breeding season (September-February), while 32·91% oestruses were observed in low breeding season (March-August). The proportion of buffaloes that exhibited FPPO during 45-90, 91-180, 181-270 and 271 days onwards was 23·57% 28.74%, 15.50% and 32.16%, respectively. 52.31% buffaloes exhibited FPPO within 6 months of calving.

* * *

The female is expected to exhibit oestrus within a couple of months after parturition. The occurrence of first heat post-partum is dependent on a number of factors such as; involution of uterus, endocrine constitution, hormonal balance, nutritional status, uterine health, mammary function, season, parity, suckling and others (Arthur *et al.*, 1982). Considerable variations have been observed in the occurrence of first post-partum oestrus and fertile oestrus in Surti buffaloes ranging from 21 to 350 days (Sane *et al.*, 1968; Rao *et al.*, 1973; Devaraj, 1982). Buffaloes calved in monsoon season and those exhibiting oestrus in winter seaso have shortest oestrus interval to post-partum (Rao *et al.*, 1973).

Luktuke and Sharma (1972) reported long post-partum ancestrus in buffaloes of organised farms as well as in rural areas to the tune of 32.82% and subfunctional ovaries were observed in 22.9% cases. However, there are very few organised farms or herds of buffaloes in India. The major part of buffalo population is owned by small and marginal farmers holding 1-3 heads per family. Because of diversity in feeding and management practices followed at this end, large number of animals exhibit either silent heat or long post-partum anoestrus under field condition, especially during the period of peak lactation and suckling.

Materials and Methods

The present study was, therefore, undertaken on a total of 755 post-partum Surti buffaloes reported in oestrus for the first time after calving and brought for AI/SHC from the field at Gujarat Veterinary College A.I. Clinic, Anand during a period of one year (1984-85). The detailed history, observations and rectal palpation findings were recorded along with the date of calving, nature of calving, date of first oestrus etc. for calculating tne first oestrus interval to post-partum. The data were tabulated

FIG-1 POST-PARTUM OESTRUS OCCURENCE IN BUFFALOES.

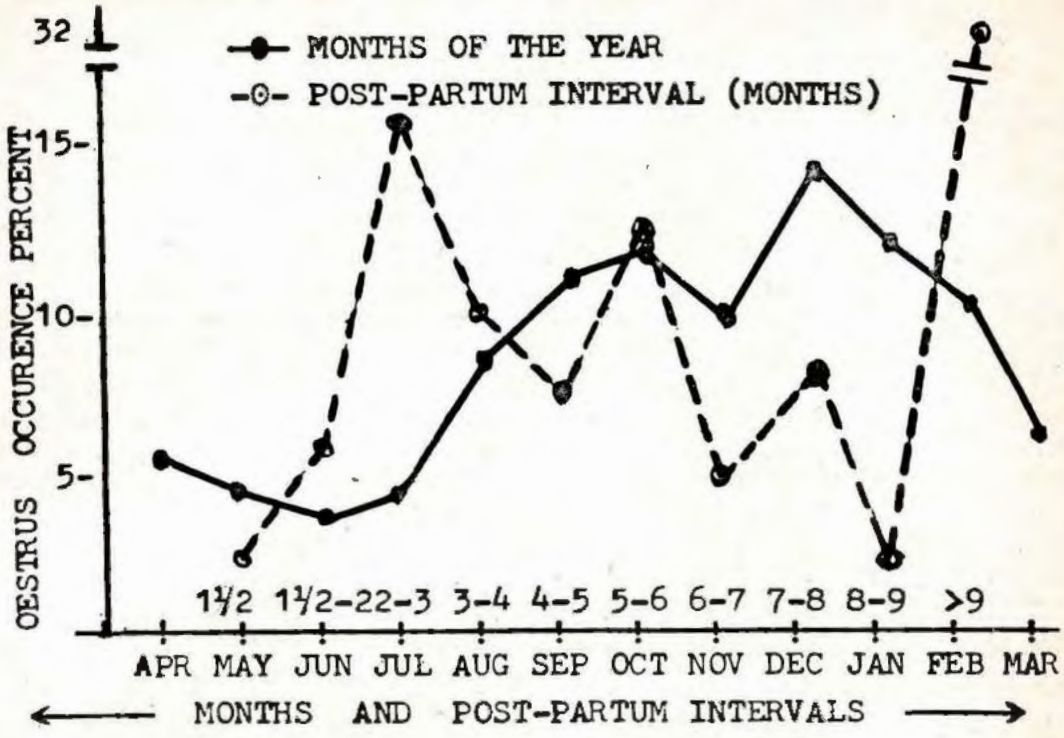

TABLE 1. Month-wise distribution of buffaloes exhibiting FPPO.

Month	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Total
No. of Buffaloes in heat	80	85	71	104	89	82	48	40	32	28	34	62	755
Percentage	10.60	11.26	9.40	13.78	11.79	10.86	6.09	5.30	4.24	3.71	4.50	8.21	100%

TABLE 2. Proportion of buffaloes exhibiting first oestrus at different post-partum intervals

Post-partum intervals (days)	45	45-60	61-90	91-120	121-150	151-180	181-210	211-240	241-270	>271	
No. of Buffaloes exhibiting FPPO	19	44	115	71	55	91	37	61	19		243
Percentage	2.52	5.83	15.23	9.40	7.29	12.05	4.90	8.08	2.52		32.16

according to month of oestrus occurrence and post-partem period for analysis.

Results and Discussion

The month-wise number and pre cent of buffaloes reported for breeding at first post-partum oestrus and the number and proportion of buffaloes exhibiing first oestrus at different post-partum interval have been presented in Table-1 & 2, respectively and in fig. 1. The results reveal that 67.09% buffaloes exhibited FPPO during high breeding season (September-February) mainly due to monsoon being calving season for this breed. While 32.91 % buffaloes expressed oestrus during low breeding season (March-August). These findings are in close agreement with the observations reported by Roy *et al.* (1972), Abhi *et al.* (1973), Derashri (1982), Kavani *et al.* (1984) and Satish Kumar (1985).

The proportion of buffaloes exhibiting first oestrus post-partum within 45 days of calving is very less (2.52%). This indirectly tells about the period of uterine involution influencing the onset of first oestrus. However, proportion of buffaloes that exhibited FPPO and bred during 45-90, 91-180, 181-270 and 271 days onwards was 23.57, 28.74, 15.50 and 32.16 percent, respectively. The majority of buffaloes (52.31%) exhibited FPPO within 6 months of calving.

The history among 32.16% buffaloes exhibiting FPPO after 270 days of calving indicated that majority of them had high milk yield with persistency in lactation and the calf was allowed to suckle for more than 6 months. This suggests that lactational stress and suckling along with under-feeding, malnutrition and negative energy balance post-partum supressed the behavioural signs of oestrus. Once the peak of lactation was over, they regained their body condition and showed signs of oestrus. These findings closely agreed with those reported by Luktuke and Sharma (1972) and Rao *et al.* (1973). However, Sane *et al.* (1968) and Devaraj (1982) reported first post-partnm oestrus and fertile oestrus in Surti buffaloes under farm conditions as 90.00 ± 8.57 days and 141.20 ± 12.7 days and 28.30 and 104.7 days respectively. These intervals are lower than the present findings.

Acknowledgement

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Incidence Of Abortion And Still Birth In Kankrej And Their Crosses.

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Prenatal calf mortalities are major sources of calf losses in both cross-bred and purebred cattle. Abortion and still birth are among the important phenomena of reproductive wastage. The present study was undertaken to find out the incidence of abortion and stillbirth in Kankrej and their halfbred with Jersey and Friesian.

The reproductive records maintained at Livestock Research Station, Sardar Krushinagar extending from Nov. '80 to Oct. '84. were analysed to find out the incidence of stillbirth and abortion in kankrej, their halfbreds with Jersey and Friesian and overall. The effect of season on incidence, post abortion and stillbirth days (post partum oestrus length) and services per conception were calculated after dividing the year into three seasons.

The results recorded in table 1 revealed that incidence of abortion and stillbirth were higher in crossbreds than native kankrej breed. The overall incidence of abortion was slightly higher than the accepted value of 2 to 5% (Roberts, 1982), and 1.3% to 1.5% (Kaikini *et al.*, 1977), but was lower than 5.98% reported by Satishkumar *et al.* (1981). The variation between the season was found to be significant. The highest rate of abortion was found during winter and the lowest incidence during summer in both the breeds. Higher rate of still birth (5 out of 10) was found during winter in kankrej and during rainy season (2 out of 3) in crossbred. It was also observed that most of the abortions and stillbirths occurred during hot hours of the day between 10.00 a.m. to 4.00 p.m. irrespective of season. In kankrej breed,

TABLE 1. Percentage of Abortion and Still Birth

Breed group	No. of observation	No. of abortion	Post-abortion oestrus days	Services per conception	No. of still birth	Post still birth days	Services per conception	Total calf losses	Post partum days	Services per conception
Kankrej	450	23/(5.1)	62.65	1.34	10/(2.2)	62	1.7	33/(7.33)	62.45	1.45
Cross-breed	66	5/(7.5)	73.40	2.00	3/(4.5)	75.66	1.66	8/(12.12)	74.25	1.87
Overall	516	28/(5.4)	68.02	1.67	13/(2.5)	68.83	1.68	41/(7.95)	64.75	1.54

Figures in parenthesis indicates respective percentage.

percentages of abortion during winter, summer and rainy seasons were 60.87%, 13.04% 26.09% respectively. Corresponding figures for crossbred were observed to be 60%, 40% and nil respectively. Overall percentages were 46.15%, 15.38%, and 38.46% respectively, for winter, Summer and rainy seasons. Kaikani *et. al.* (1977) reported incidence of 1.1% and 2.3% of stillbirth in Sahiwal and Murrah breeds respectively which were similiar to the present results of Kankrej breed and overall average. Among crossbreds Satishkumar *et al.* (1981) reported 6.96% stillbirth and Rao (1982) observed 4.1% cases in crossbreds which were in close accordance with present results of crossbreds. An increase in the abortion rate from 1.58% to 9.63% with increase in Jersey inheritance was reported by Ramamohana Rao *et al.* (1970). Amble and Jain (1967) found the rate of abortion, stillbirth and premature calving to be 8% in Sahiwal 1% in 5th and 21% in 31/32 Holstein grades.

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Crenellation Pattern And Semen Quality In Cross-bred Bulls

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ABSTRACT

The crystallization/crenellation pattern of semen drop was studied in 192 ejaculates from 8 cross-bred bulls and 6 ejaculates from a crossbred bull with testicular hypoplasia. The study covered a period of one year divided into cold, hot and wet seasons. The mean crenellation pattern score observed was 2.16 ± 0.05 (scale 1-3). There was highly significant difference in crenellation pattern between bulls and between bulls × seasons inderactions. However, the effect due to season was nonsignificant. The crenellation pattern score was 2.29 in bulls with excellent sexual function (3), 2.09 in normal sexual function (4) and 1.21 in poor sexual function (2). The pattern was signi- ficantly and positively correlated with colour & consistency (0.398), mass activity (0.363), individual sperm motility (0.186), sperm concentration/ml (0.475), sperm concentration/ejaculate (0.249) and live sperm percent in cold shock test (0.141). The pattern was significantly but negatively correlated with dead sperm percent due to cold shock test (−0.140). The test proved to be inexpensive, simple, rapid and reliable for evaluation of semen quality.

* * *

There has been no single reliable test for semen evaluation precisely & accurately within a short period of time. However, visual appraisal of initial mass activity and motility after dilution, through microscope and estimation of sperm concentration are simple and rapid tests routinely used by almost all A.I. centres/semen banks (Zemjanis, 1970). With the advent of differential interference contrast optics, it has been possible to assess the quality of live and unstained spermatozoa (Saacke and Marshall, 1968).

The literature on precipitation/crystallization/crenellation pattern of different dried semen samples for evaluation of semen quality is scarce (Verma *et al.*, 1982). Blom (1972) reported that crystallization of semen for evaluation of seminal plasma total solids in bovine to be indirectly helpful to evaluate the semen quality provided if all the accessary secretions have contributed to the ejaculate in a normal way. Low value (<5%) of total solids without crystallization pattern indicated incomplete ejaculate or vesiculitis. The present study was undertaken in cross-bred bulls to assess whether the crenellation pattern of semen drops on drying is reliable for evaluation of semen quality.

Materials and Methods

The study was undertaken on 9 cross-bred bulls (4 K×J, 4 K×HF & 1 K×HF×HF) aged 4-5 years, maintained at the Department of Gynaecology, Gujarat Veterinary College, Anand, during a period of one year. The year

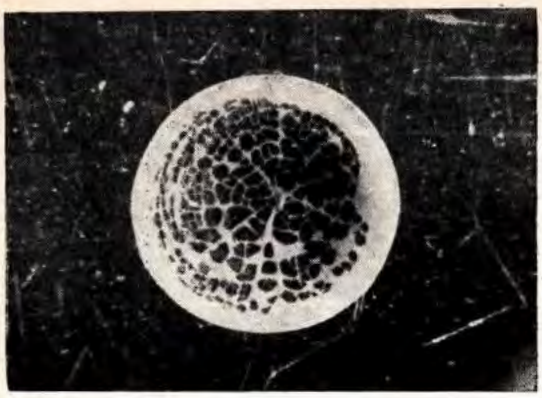

Fig. 1 Macroscopic View of (48×) Excellent Crenellation Pattern.

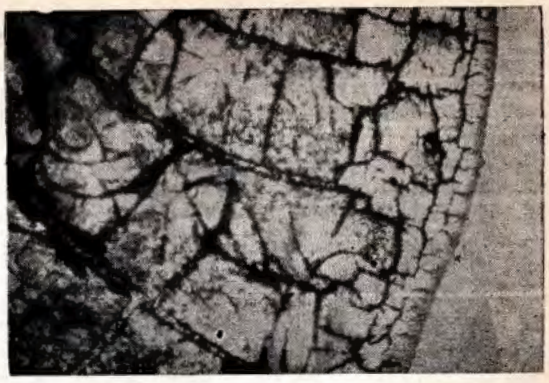

Fig 2 Microscopic View (300×) of Excellent Crenellation Pattern.

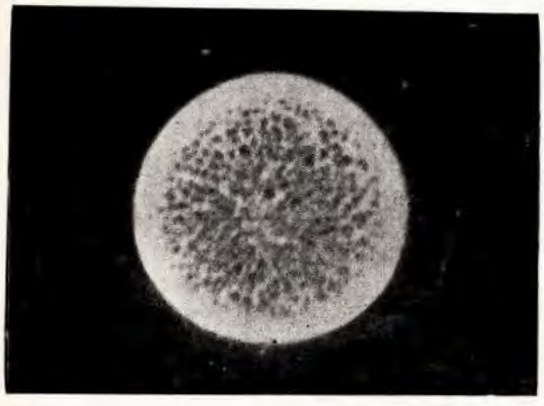

Fig. 3 Macroscopic View (33×) of Medium Crenellation Pattern.

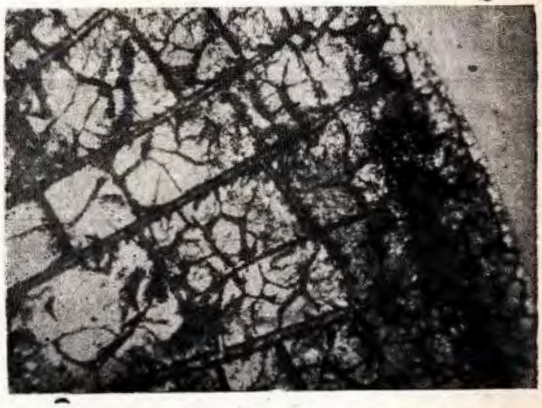

Fig. 4 Microscopic View (×300) of Medium Crenellation Pattern.

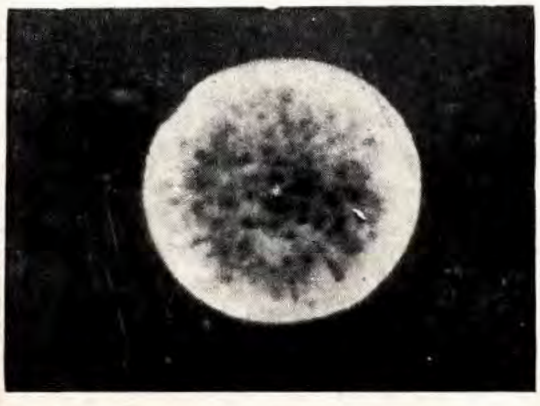

Fig. 5 Macroscopic View (33×) of Poor Crenellation Pattern.

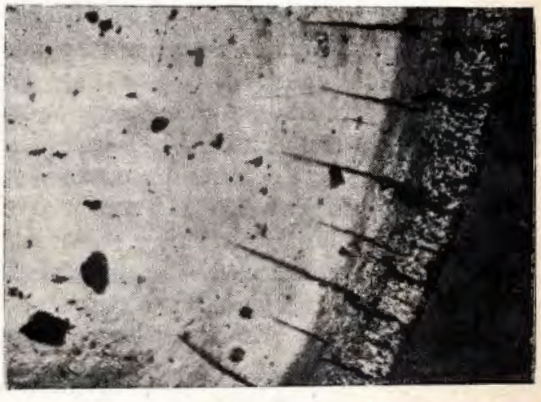

Fig. 6 Microscopic View (300×) of Poor Crenellation Pattern.

TABLE 1. Crenellation Pattern of Semen in Crossbred Bulls (Scale 1-3).

Sr. No.	Name of the Bulls	Seasons			Bull Average**
		Cold	Hot	Wet	
1.	Zanjar K×J	1.88±0.23	2.38±0.26	2.38±0.26	2.13±0.16
2.	Tara K×HF	1.75±0.25	1.88±0.23	2.50±0.27	2.04±0.17
3.	Dhol K×J	2.38±0.18	2.63±0.18	1.88±0.23	2.29±0.13
4.	Tara K×HF	2.38±0.26	2.00±0.27	2.63±0.18	2.33±0.14
5.	Kajal K×J	2.38±0.18	2.13±0.23	2.25±0.31	2.25±0.14
6.	Singar K×HF	2.38±0.18	1.75±0.16	2.25±0.16	2.13±0.11
7.	Kabar K×HF	2.00±0.33	2.13±0.23	1.88±0.30	2.04±0.17
8.	Rosy K×J	2.50±0.19	1.63±0.18	2.13±0.23	2.08±0.13
9.	Deolo K×HF×HF	—	—	—	1.33±0.37
Season Average		2.20±0.08	2.36±0.34	2.23±0.09	2.16±0.05

TABLE 2. Critical Difference tests.

Sources	SEm	C.D.	C.V.%
Bulls	0.24	0.67**	55.28
Seasons	0.15	NS	
Interactions	0.42	1.16**	

NS = Nonsignificant; ** Significant at 1% level.

was divided into three seasons viz., cold (Nov-Feb), hot (Mar-Jun) and wet (Jul-Oct). The semen collections were made between 7 to 8 a.m. at weekly interval, using artificial vagina and a bull of same species as teaser. All the bulls had normal serving ability and serving behaviour. A total of 192 biweekly collected ejaculates were utilized for the present study. In addition, 6 ejaculates collected twice a week interval from bull Deolo-K×HF×HF- with testicular hypoplasia were also studied. Semen samples were evaluated according to standard procedures for volume, colour & consistency, pH, motility, sperm count, percent live-dead & abnormal sperms (Campbell *et al.*, 1953), and cold and hot shock tests (Herman and Maddan, 1953). Crenellation pattern of semen was studied immediately after collection by drying a drop of semen on a clean glass slide at room temperature. The pattern was graded according to Verma *et al.* (1982) with slight modification. The scores 1, 2 and 3 were assigned for poor, medium and excellent crenellation pattern, respectively. The data so recorded were statistically analysed according to Snedecor and Cochran (1967) and the crenellation patterns were correlated with semen characteristics.

Results and Discussion

The mean scores of crenellation patterns during cold, hot and wet seasons have been presented in table-1 and results of critical difference tests in table-2.

TABLE 3. Correlations between Crenellati on pattern and seminal characteristics

Sr. No.	Semen Characteristics	Crenellation Pattern (correlation, r)
1.	Libido	—0.094
2.	Thrust	—0.053
3.	Reaction time	0.018
4.	Ejaculate volume	0.072
5.	Colour & consistency	0.398*
6.	Seminal PH	0.071
7.	Mass motility	0.363*
8.	Individual motility	0.186*
9.	Optical Density	0.436*
10.	Sperm Concentration/ml	0.475*
11.	Sperm concentration/ejaculate	0.249*
12.	Live sperm percent	0.026
13.	Dead sperm percent	—0.025
14.	Abnormal sperm percent	—0.028
15.	Dead sperm % in cold shock test	—0.140*
16.	Live sperm % in cold shock test	0.141*
17.	Dead sperm % due to hot shock test	—0.111
18.	Live sperm % due to hot shock test	0.121

* Significant at 5% level.

The mean crenellation pattern score observed was 2.16 ± 0.05. There was a highly significant difference in crenellation patterns observed between bulls under study. The score/crenellation pattern was 2.29/excellent in three bulls with excellent sexual function, 2.09/medium in four bulls with normal sexual function and 1.21/poor in two problem bulls (Fig. 1 to 6). However, the effect of seasons was found to be nonsignificant. There was highly significant difference for bulls × seasons interactions in crenellation pattern. In problem bull 'Deolo', the crenellation pattern observed was poor (1.33 ± 0.37 score). The crenellation patterns in relation to semen evaluation have been studied by Blom (1972) and Verma *et al.* (1982). These authors reported that the characteristic pattern assumed by semen drops on drying showed close correlation with mass activity of semen. They further stated that the test was simple, rapid inexpensive which could be routinely used for semen evaluation even under ordinary field conditions.

The crenellation pattern of semen in the present study showed significantly positive correlations with most of the semen characteristics except live sperm% in cold shuck test (Table-3). These correlations indicated that the test is simple, rapid and reliable for semen evaluation.

Acknowledgement

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IJAR 7: 1: 119-123, 1986

Seminal Fructose And Reaction Time In Cross-bred Bulls

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ABSTRACT

Eight cross-bred bulls (4 K×J & 4 K×HF) aged 4-5 years, were andrologically investigated at the Department of Gynaecology, Gujarat Veterinary College, Anand for a period of one year. The year was divided into cold, hot and wet seasons. The bulls were on weekly once collection schedule but a total of 192 biweekly collected ejaculates were used for the present study. The objective was to study the effect of bulls, seasons and bulls×season interaction on reaction time and initial seminal fructose content.

The overall mean reaction time and initial fructose content were 135.00±9.41 sec. and 540.9±19.38 mg%, with mean values in cold, hot and wet seasons as 141.56±19.02, 125.15±11.88 & 135.20±17.43 seconds and 579.34±39.26, 392.18±28.09 & 631.51±25.70 mg%, respectively. The effect of bulls and seasons were statistically nonsignificant. However, bulls×season sinteraction significantly ($P<0.05$) affected the reaction time. The comparison between mean reaction time and initial fructose content revealed that as the reaction time increased, there was reduction/decline in the levels of initial fructose content. This showed the existence of a negative correlation between these two traits. Seminal fructose levels indirectly communicated the levels of testosterone in the bulls under study.

* * *

Cross breeding of indigenous dairy breeds of cattle using exotic germ plasm has been accepted as an effective method for bringing about a rapid genetic improvement in the Indian cows leading to not only increased milk production but also meat production. In the process of evolving and perpetuating new breeds or strains of cross-bred cattle with different levels of exotic germ plasm through A.I., use of cross-bred bulls in the breeding programme has become imminent. Very little information is available on the various aspects of reproduction and seminology in cross-bred bulls (Mathew, 1974; Rao and Rao, 1978 & 1979). The present investigation was, therefore, undertaken to study the effect of bulls, seasons and bulls-seasons interactions on the reaction time and initial seminal fructose content in cross-bred bulls.

Materials and Methods

Andrological investigations were undertaken on eight crossbred bulls (4 K×J & 4 K×HF) aged 4 to 5 years, at the Department of Gynaecology, Gujarat Veterinary College, Anand. The bulls were maintained under identical managemental conditions. The investigation was carried out for a period of one year commencing from November, 1983 to October, 1984. The year was divided into three seasons viz., cold (Nov-Feb), hot (Mar-Jun) and Wet (Jul-Oct). One

TABLE 1. Initial fructose and reaction time in cross-bred bulls during different seasons

Sr. No.	Name and No. of bulls	Breed	Seasons	Reaction time (sec.) Mean±S.E.	Initial fructose (mg/100 ml) Mean±S.E.
1.	Zanjar 0—25	K×J	I Cold	116.25± 17.62	622.23±141.49
			II Hot	223.75± 3.85	377.09± 76.25
			III Wet	269.13± 77.91	672.21± 44.05
			IV Average	211.25± 30.01	557.18± 59.51
2.	Tara 0—28	K×HF	I	150.00± 9.26	646.54±108.58
			II	160.00± 4.10	277.09± 98.81
			III	245.00± 53.39	497.21± 55.55
			IV	170.00± 21.43	473.61± 58.183
3.	Dhol 0—26	K×J	I	116.25± 11.49	605.55±108.98
			II	102.50± 4.12	338.19± 74.00
			III	141.25± 17.05	706.93± 70.05
			IV	120.00± 7.47	550.22± 57.53
4.	Tara 0—29	K×HF	I	376.25±126.60	559.04±104.87
			II	211.25± 59.66	441.20± 64.44
			III	208.75± 57.77	568.08± 77.68
			IV	265.42± 50.92	513.44± 48.66
5.	Kajal 9—23	K×J	I	95.00± 13.63	515.96±116.71
			II	100.00± 14.64	532.64+ 82.43
			III	62.50± 13.98	752.78± 67.01
			IV	85.83± 8.51	600.46± 55.08
6.	Singar 9—19	K×HF	I	102.50± 9.01	623.63± 93.94
			II	55.00± 10.86	435.36± 73.05
			III	45.00± 8.02	588.20± 69.12
			IV	67.50± 7.35	549.06± 47.03
7.	Kabar 9—20	K×HF	I	101.25± 9.15	577.09±110.77
			II	66.25± 9.99	430.56± 70.49
			III	52.50± 9.40	684.03± 75.61
			IV	73.33± 6.78	445.83± 47.77
8.	Rosy 8—14	K×J	I	120.00± 8.86	484.73± 85.70
			II	82.50± 16.12	333.35± 93.21
			III	57.50± 12.92	582.65± 96.37
			IV	86.67± 8.94	466.90± 55.01
9.	Deolo —	K×HF×HF	Average	105.00± 29.25	319.45±136.14
	Overall		Cold	141.56± 19.02*	579.34± 37.26
			Hot	125.15± 11.88*	392.18± 28.09
			Wet	135.20± 17.43	631.51± 25.70
			Average	135.00± 9.41	540.59± 19.38

* Significant at 5% level

Problem bull Deolo-K×HF×HF (total testicular hypoplasia)—belonging to a private dairy farm was also included in the study.

The semen collections were made between 7 to 8 a.m. at weekly interval, using artificial vagina and a bull of same species as teaser. The serving ability, serving behaviour and detailed seminal attributes were evaluated and recorded based on 192 biweekly collected ejaculate, 24 from each of 8 bulls. Another 6 ejacu-

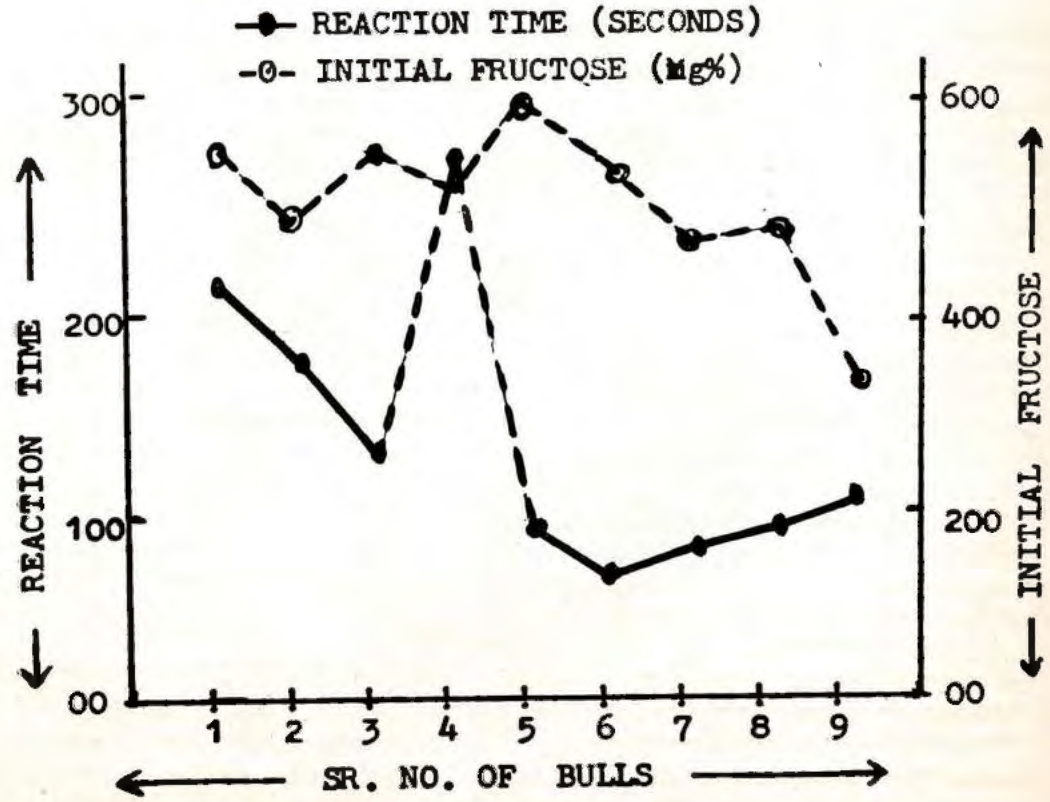
TABLE 2: Critical difference tests for bulls, seasons and interaction effects.

Sources	Reaction time			Initial fructose		
	SEm	CD	CV%	SEm	CD	CV%
Bulls	4.16	NS	15.11	28.90	NS	26.19
Seasons	2.55	NS		17.70	NS	
Interactions	7.21	19.97*		50.06	NS	

* Significant at 5% levels
NS Nonsignificant.

lates were obtained from bull Deolo under twice a week collection schedule for investigations. The reaction time was noted as time interval (in seconds) between the bull was in proximity of the teaser till donation of semen. Initial fructose content in neat semen was assayed according to method described by Mann (1948). The data were analysed according to standard statistical proce-

FIG-1 INITIAL FRUCTOSE & REACTION TIME IN CROSS-BRED BULLS.



dures (Snedecor and Cochran, 1967) using ICL-2950 computer.

Results and Discussion

The reaction time and initial fructose content of semen in cross-bred bulls during different seasons and the critical difference tests for the effect of bulls, seasons and bull×season interactions have been presented in table 1 & 2, respectively.

The mean reaction time between bulls varied from 67.50±7.35 to 265.42±50.92 sec. with an overall mean of 135.00±9.41 sec. Similarly initial fructose content also varied from 445.83 ±47.77 to 600.46± 55.08 with a mean of 540.59±19.38 mg %. The differences between bulls for these characters were statistically non-significant. The effects of seaons were also non-significant. However, the initial fructose and reaction time were apparently low in hot and wet seasons, respectively. The overall mean values for bulls×seasons interaction in reaction time and initial fructose were apparently different for both the traits. However, the differences for reaction time were only statistically significant but not for initial fructose.

Doicheva *et al.* (1980) observed non-significant effect of sasons on initial fructose content. However, Choudhary and Sadhu (1983) reported that the initial fructose content was highly significantly affected by seasons being lowest in summer. Ali. *et al.* (1983) reported the average reaction time in bulls to be 114.6±6.8 seconds. They further stated that the bull effect was significant but not the season effect. Tomar and Gupta (1984) reported that there was no significant difference in reaction time between seasons in Hariana bulls. Rao and Rao (1975) found the fructose content in semen of Tharparkar and Jersey bulls to be 625.87±55.20 and 648.05±48.81 mg %, respectively. While Bhosrekar *et al.* (1984) observed 416.152±6.53 and 379.85±29.86 mg % fructose in HF and Jersey bulls, respectively. These findings are in agreement with the present findings.

The comparison of mean reaction time and initial fructose content in semen of nine cross-bred bulls has been illustrated in Fig. 1. It can be seen that as the reaction time increased, there was reduction/decline iu the levels of initial fructose coutent in semen samples. However, the bull Tara 0-29 did not show this trend. The alteration in reaction time in this bull was due to disturbed serving behaviour. The trend indicated the existence of a negative correlation between these two characters. These findings are in agreement with those reported by Abdou *et al.* (1978) in HF and buffalo bulls. They have reported that the initial fructose in HF and buffalo bulls was significantly & negatively correlated with reaction time (−0.22 & −0.35, resp.). The fructose is an important metabolite for bovine spermatozoa. The seminal fructose value gives a useful indication of the fertilizing ability of bulls. The fertile bull semen showed significantly higher concentration of fructose than infertile bull-Deolo. Increase in the fructose level will indicate more metabolic activity of sperm. The seminal fructose level indirectly communicated the levels of testosterone and related steroids in the bulls under study. However, their levels could not be assayed for want of RIA facilities at this end.

Acknowledgement

The authors are grateful to Dr. M.R. Patel, Principal, Gujarat College of Veterinary Science and Animal Husbandry, Anand for the facilities, to

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Different Types Of Scrotii And Their Relationship With Semen Characteristics Of Murrah Buffalo Bulls

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ABSTRACT

In Murrah buffalo bulls oblong type of scrotum was found to be the most common variety (69.77%) followed by square (20.93%) and overlapping (9.30%) types. The semen characteristics did not vary significantly among buffalo bulls with different shapes of scrotii.

• • •

Very little is known about the relationship between the type of scrotum and the semen characteristics. Hence the present study was undertaken with an aim to find out the relationship between the type of scrotum and semen characteristics in Murrah buffalo bulls.

Materials and Methods

Forty three Murrah buffalo bulls aged between 53-163 months maintained at Frozen Semen Bank, Indo-Swiss Project, Visakhapatnam were examined for the type of scrotum and classified as oblong, square and overlapping types. A total of 129 ejaculates obtained with the help of artificial vagina were studied for semen characteristics.

Results and Discussion

In Murrah buffalo bulls oblong type of scrotum was found to be the most common variety (69.77%) followed by square type (20.93%) and overlapping type (9.30%) (Fig.). The median raphae of the scrotum was observed to be on the right side, left side and exactly on the centre of the scrotum respectively in 2.17, 76.09 and 21.74% of the buffalo bulls inspected. Similar observations regarding types and incidence of scrotii were also recorded by Bedi (1980) in buffalo bulls.

The overall values of different semen characteristics were: ejaculate volume 3.71 ± 0.19 ml; initial motility $79.54 \pm 0.88\%$; concentration of spermatozoa $1131.12 + 41.07$ millions per ml; Live sperm count $87.67 \pm 0.49\%$; Sperm head abnormalities $4.12 \pm 0.16\%$; Loose heads $1.90 \pm 0.32\%$; mid piece abnormalities $1.12 \pm 0.07\%$; Proximal protoplasmic droplets $1.31 \pm 0.11\%$ and tail abnormalities $5.30 \pm 0.42\%$.

The seminal attributes viz., ejaculate volume, mass activity, initial motility, sperm concentration, live sperm percentage and spermiogram did not differ significantly among Murrah buffalo bulls with different types of scrotii. These observations were in agreement with those of Bedi (1980).

The incidence of different sperm abnormalities recorded in the present study was within the range stipulated for normal

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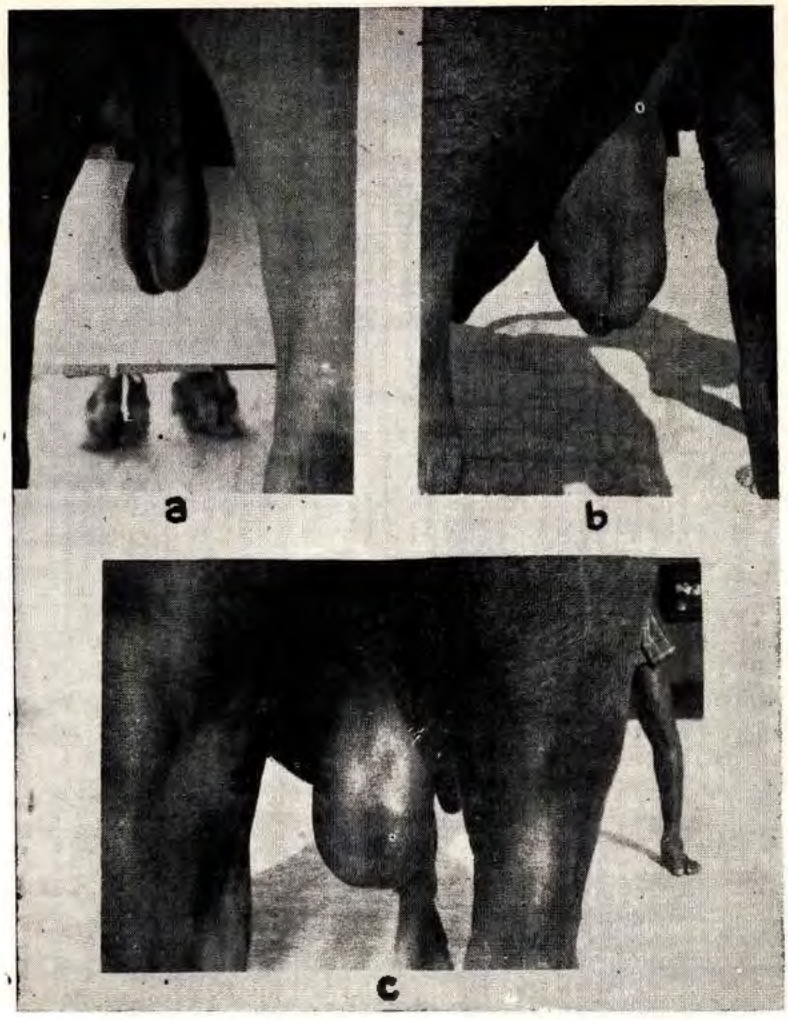

Fig. Photoplate showing different types of scrotii in Murrah buffalo bulls

a = Oblong type of Scrotum
b = Overlapping type
c = Square type

fertile bulls (Blom, 1950 and Rao, 1971) and buffalo bulls (Gopalakrishna and Rao, 1978).

Since none of the seminal attributes significantly differed among animals with different types of scrotii, all the three types might be considered as abnormal variants of the scrotal shape and bulls with such types of scrotii can be selected and used for breeding purposes.

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Correlations Between Seminal Characters And Fertility In Surti Buffalo Bulls

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ABSTRACT

Correlation studies have been reported in Surti buffalo bulls between semen characters and fertility based on 500 fresh artificial insemination and 792 ejaculates.

Correlation studies between fertility and seminal characters revealed highly significant positive correlations for sperm concentration, significant positive correlations for live sperm per cent and highly significant negative correlation for abnormal sperm per cent in Surti buffalo bulls.

* * *

It is considered that no single test for semen evaluation is adequate to predict the fertility while a combination of tests might yield useful information. The relationship between semen characters and fertility in zebu bull has been reported by Swanson and Herman (1944), Stone *et al.* (1950) and in Murrah bulls by

TABLE 1: ANOVA

Sr. No.	Semen Characters	Sources of variation	D.F.	S.S.	M.S.S.	Cal. 'f'	Table 'f'
1.	Volume/ml	(a) Between seasons	1	4.95	3.957		
		(b) Within season	412	358.43	0.869**	5.698**	3.86
2.	Mass activity	(a)	1	0.80	0.803		
		(b)	412	31.48	0.076	10.516**	3.86
3.	Motility	(a)	1	129.51	129.510		
		(b)	412	12164.11	29.526	4.386**	3.86
4.	Sperm Con./ml.	(a)	1	1003001.00	1003001.00		
		(b)	412	37852689.00	91875.458	10.917**	3.86
5.	Live sperms percent	(a)	1	66.49	66.490		
		(b)	412	11833.19	28.721	2.315NS	3.86
	Abnormal sperms						
6.	(a) Head	(a)	1	0.33	1.210		
		(b)	412	498.60	0.322	3.188NS	3.86
7.	(b) Tail	(a)	1	0.33	0.322		
		(b)	412	498.60	1.210	0.2749NS	3.86
	(c) Total	(a)	1	5.47	5.479		
		(b)	412	1371.00	3.327	1.646NS	3.86

** Highly significant; NS = Non significant.

TABLE 2: Correlations between semen characteristics and fertility in buffalo bulls.

Bulls	Young (10)				Prime (23)				Adult (11)			
	Motility	Sperm conc. /ml × 10^6	Live sperm percent	Abnormal sperm percent	Motility	Sperm conc. /ml × 10^6	Live sperm percent	Abnormal sperm percent	Motility	Sperm conc. /ml × 10^6	Live sperm percent	Abnormal sperm percent
Conceptions per cent	NS 0.0924	* 0.7491	0.2481	** 0.7653	NS —0.0464	** +0.7276	* +0.5021	NS 0.0227	NS —0.4961	NS —0.0984	* 0.6133	NS 0.3679
Motility	—	NS 0.2371	NS 0.2319	NS 0.3314	—	NS 0.1004	NS —0.1213	NS 0.2313	—	0.0811	NS —0.2985	NS —0.0560
Sperm conc./ ml × 10^6	—	—	NS 0.03013	0.4081	—	—	NS 0.2208	NS 0.0825	—	—	NS —0.2933	NS —0.0740
Abnormal sperm percent	—	—	—	NS 0.1401	—	—	—	NS —0.0569	—	—	—	NS 0.0800

** Highly significant,

* significant,

NS = Non significant

Gopalakrishna and Ramamohana Rao (1979). The present paper deals with the results of studies on correlations between semen characters and fertility in Surti buffalo bulls.

Materials and Methods

The data (Madhu Rao, 1976) available on 500 fresh artificial inseminatinos with semen from 44 bulls were utilized. A total of 792 ejaculates (nine first and nine second) in 44 buffalo bulls collected during different periods were evaluated for semen characters by following standard semen laboratory procedures.

All the forty four bulls studied were categorised into three groups according to their ages, viz., young bulls upto 3 years, prime bulls 4 — 7 years, and adult bulls 8-12 years.

Statistical analysis was done by using IBM-1620 computer data processing machine and the methods followed according to Snedecor and Cochran (1967).

Results and Discussion

The results of anlysis of variance for the various semen characters studied have been presented in Table-1.

From the Table-1 it is evident that highly significant variations for the seminal characters, viz., volume/ml, mass activity, motility after dilution and sperm concentration existed between the seasons and within the season. However, the live sperm percentage, head abnormality, total abnormality, and tail abnormality did not vary significantly.

The reuslts of correlation studies between semen characteristics and fertility in young, prime and adult group of buffalo bulls have been presented in Table-2.

From Table-2 correlation studies between fertility and seminal characters revealed that highly significant positive correlations existed for sperm concentration, significant positive correlations for live sperm per cent and highly significant negative correlation for abnormal sperm per cent in young, prime and adult group of buffalo bulls.

It was observed that sperm concentration, abnormal sperm per cent and live sperm per cent consideration was more important in young and prime bulls and live sperm per cent in adult bulls. Therefore, in evaluation of semen samples, estimation of sperm concentration, counts of abnormal sperm and live sperm could be used for knowing their fertility ability.

Similar correlation studies were reported by Basant Singh *et al.* (1968) and Tomar and Basant Singh (1970) in Murrah buffaloes bulls. The results in the present correlation studies agreed with their findings.

Acknowledgement

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Studies On Equilibration Period, Thawing Time & Temperature And Viability Of Buffalo Semen

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ABSTRACT

The equilibration period for freezing buffalo bull semen varies from lab to lab, the average being 5 to 6 hours. The village level inseminators usually judge the temperature of water for thawing straws by finger-dipping and without giving due consideration to the thawing time, which can be highly erroneous especially during different seasons. During the present investigation, the pre-freeze and post-freeze sperm motility of buffalo bull semen for four equilibration periods viz., 1 hr, 2 hrs, 3 hrs and 4 hrs while post-thaw motility for three thawing temperatures viz., 20°C, 34°C and 42°C with the thawing time for each of the temperature being 30 sec., 120 sec. and 300 sec. were studied. The analysis of variance and the comparison of the means with LSD revealed that there was no significant difference in the post-freeze-thawed motility at 0 hr and 24 hrs, when the equilibration period was either 3 hrs or 4 hrs. At higher thawing temperature, the post-thaw motility rose steeply but also dropped down suddenly with the increase in time period. At 20°C the post-thaw motility was poor. The thawed semen at 34°C took on an average 27.8 mts, whereas at 42°C it took 16.1 mts to become completely flat.

* * *

The advent of artificial insemination has considerably enhanced our capacity to propagate the superior germ-plasm. Its application has been enormously increased with the introduction of frozen semen technique. Since the reporting of the basic method of freezing spermatozoa by Polge in 1949, there has been considerable improvement in freezing technology and its application in artificial insemination work. Fertility studies to determine the optimal equilibration time have been minimal with straws (Eapen, 1961; Jondet, 1967 and 1972). Crabo *et al.* (1981) reported equilibration time of 7 hrs to give better results than that of 5 hrs, while freezing the semen of Nili-Ravi buffalo bulls. However Wiggin and Almquist (1975) found no difference between 1/2 hr and 2 hrs glycerol equilibration time and concluded that the best glycerol equilibration time has not yet been established. Similarly, the procedure for thawing semen in straws has also been one of the most important factor influencing the spermatozoal viability. (Aamdal and Anderson, 1968 a, b; Robbins *et al.*, 1972, 1973, 1976; Crabo *et al.*, 1979 and Mathew, 1984). The thawing rates and procedure have varied considerably (Bean, 1972; Rugg and Pickett, 1977; Davidovic *et al.*, 1971). As per Pickett *et al.* (1980) elevated thawing temperatures can cause

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a transient stimulation of motility followed by a rapid decline in survival. This study was undertaken on buffalo bull semen to ascertain the effect on motility of (a) variable equilibration time (1 hr, 2 hrs, 3 hrs, 4 hrs) (b) variable thawing time and temperature and (c) viability of spermatozoa at variable thawing time and temperature usually encountered under field conditions.

Materials and Methods

The study was carried out on 6 randomly selected Murrah buffalo bulls. Once a week collections were taken from each of the bulls over a period of six consecutive weeks. All the normal standards of asepsis and hygiene were strictly adhered to during collection, evaluation and processing of the semen. The collected semen was evaluated for colour, volume, density, mass activity, initial motility, live-dead count and sperm concentration. Based on the initial evaluation, substandard ejaculates, if any were rejected. The dilution rate was based on sperm concentration keeping 60 millions live spermatozoa per ml of diluted semen. The Tris-egg yolk-glycerol dilutor was added in 2 equal parts. The first half of the diluent containing 3% glycerol was added to each ejaculate as initial dilution and transferred to the cold handling cabinet maintained at 5°C. Within 1½ hr, the semen was cooled to nearly 5°C. The second half of the diluent containing 9% glycerol (maintained at 5°C) was then added in two equal portions at 10 minutes interval. The equilibration period (EP) in this study started after the second half of the diluent was completely added, thereby bringing the final glycerol level to 6%. Filling of the french straws (0.54 ml capacity, 135 mm in length and 2.8 mm in diameter) was accomplished by using automatic filling sealing machine (IMV. France) kept in cold handling cabinet, The sealing was accomplished by ultrasonic vibrations applied to the open end of the straws resulting in molecular changes and consequent sealing. At the end of every equilibration period, prefreeze motility was determined by taking 2 starws, whereas 6 straws for every bull and each EP were frozen each time for determining post freez 0 hr and 24 hrs motility. Freezing was accomplished by using pre-cooled ramps, racks etc. and wide mouthed LN_2 container (320 L capacity). For thawing, a water bath was constantly maintained either at 20°C or 34°C or 42°C. The thawing time was controlled by using a stop watch as either 30 sec or 120 sec or 300 sec. At each of the thawing temperature and time, the motility recovery rates were recorded for all the six bulls semen during the six weekly collections. Further, at 34°C or 42°C thawing temperature, the viability of the frozen semen was ascertained by recording motility results every 300 secs till all the sperm were found dead.

Results and Discussion

The difference in motility rating at 0 hr and 24 hrs after freezing for 4 different equilibration periods were highly significant ($P<0.01$). On arcsin transformation, the mean values were as given in Table 1.

The Table 1 indicated that there was no significant motility difference seen at 24 hrs after freezing when the E.P. was either 3 or 4 hrs. These results were substantiated by partitioning the treatments and studying (a) pre-freeze and 0 hr post freeze motility difference (b) prefreeze and 24 hrs post freeze motility difference (c) 0 hr and 24 hrs post freeze motility difference for each of the 4 different E.P. The results obtained have been summarised in Table 2.

TABLE 1: Motility Rating

E.P. (hrs.)	1	2	3	4
$\bar{X}$ (0 hr, LSD = 3.39)	43.92	50.00	51.83	52.78
$\bar{X}$ (24 hrs, LSD = 3.82)	40.33	46.25	50.99	52.83

TABLE 2: Anova

Motility differences between / E.P.	1 hr	2 hrs	3 hrs	4 hrs
Pre-freeze and 0 hr	69.92**	35.90**	16.53**	4.08*
Pre-freeze and 24 hrs	125.81**	52.86**	16.50**	2.91NS
0 hr and 24 hrs	9.86**	4.26*	0.30NS	0.005NS

The significant differences gradually changed as E.P. increased to 4 hrs. At 3 hrs E.P. and 4 hrs E.P. there was no significant difference between motility observations at 0 hr and 24 hrs after freezing. It could therefore be implied that it is possible to reduce the equilibration period to 3 hrs for freezing buffalo semen. It would ofcourse be desirable to study fertility results and correlate them with semen equilibrated for 3 hrs ptior of freezing.

Further, to determine the optimal thawing time and temperature under field condition, post thaw motility rates were determined for each of the thawing time (30 sec, 120 sec and 300 sec) and temperature (20°C, 34°C and 42°C) combination. On analysis of variance, it was revealed that there was a highly significant difference ($P<0.01$) amongst the collections made from the different bulls, indicating thereby that there existed an inheriant genetic difference within the bulls tried in the experiment. Similarly the different thawing temperature and time did have a highly significant difference ($P<0.01$) amongst themselves. On calculating the motility means (using arcsin transformation) of all 36 observations for each of the thawing time and temperature, the following results were obtained and are presented in Table 3 with LSD being 4.95.

It was indicated that at 20°C, the post thaw motility irrespective of thawing time was low. There was almost no difference in post thaw motility at 34°C/30 sec and

TABLE 3: Thawing temperature (°C)/time (seconds):

20/30	20/300	20/120	34/30	42/300	42/30	34/300	34/120	42/120
23.7	26.4	29.1	41.0	44.3	47.6	50.2	50.6	51.7

42°C/300 sec. Similarly, the post thaw motility at 42°C/300 sec and 42°C/30 sec fell under the same category but the motility was better at 30 sec. The post thaw motility at 42°C/30 see, 34°C/300 sec, 34°C/120 sec and 42°C/120 sec also fell under the same category. It was also evident that motility was higher at 42°C/120 sec than at 42°C/300 sec thereby indicating that at higher temperature, eventhough the motility rose steeply, a fall was observed with the increase in time. Under field conditions, where lay inseminators usually carry out inseminations without strictly following thawing temperature/time combination, a thawing temperature of 34°C for 120 sec can be safely advocated while using buffalo semen. To substantiate these findings, viability of thawed semen at 34°C and 42°C was further carried out. On considering the average time taken for the normally motile (range 40 to 80%) thawed semen to become flat, it was found that at 34°C, thawing temperature, it took 27.8 minutes to become totally flat which was significantly more when compared to 42°C, where it took 16.1 minutes to become totally flat.

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IJAR 7: 1: 134-136, 1986

Fertility Following Heterospermic Inseminations In Surti Buffaloes

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ABSTRACT

In the field level fertility trials in Surti breed of buffalo, the frozen semen from four different Surti bulls (B1, B2, B3 and B4) was used. 1014 buffaloes/heifers were inseminated at four different field A.I. Centres by lay inseminators. 831 animals were followed for pregnancy diagnosis. The fertility rate achieved was 45.79%, 40.33* %, 43.55* % and 44.94 % respectively. The pooled semen (P) prepared out of mixing split ejaculates of all the four bulls under study was utilized for fertility trials at the same centres simultaneously and 291 buffaloes/heifers were inseminated. Out of these, 238 wee foltlowed for pregnancy diagnosis. The fertility rate achieved was 49.58%, which showed significant difference in two bulls, out of total four bulls studied for fertility.

* * *

Literature available is scanty on fertility trials using heterospermic inseminations in buffaloes. Though the studies on mixing of seminal plasma of two different bulls and its effect on progressive motility of spermatozoa have been carried out, but actual usage of heterospermic inseminations to improve fertilizing capacity of semen at field level has not been recorded. Therefore, this study was carried out to understand the role of heterospermic inseminations towards increasing fertility.

Materials and Methods

Fertility trials were conducted at field level in buffaloes of Surti breed using frozen semen. The semen from four Surti buffalo bulls was obtained in twice a week collection schedule, using artificial vagina. The semen was collected in sterilized collection tube and evaluated as per the standard techniques for volume, colour, consistency, mass activity (Herman and Madden, 1953), live and abnormal spermatozoal percentage (Hancock, 1951) and individual progressive motility of spermatozoa. Semen samples with optimum quality (minimum+++ mass activity and 70% motility) were retained for further processing. Each semen ejaculate, thus selected was split into two parts. One part of each individual bull's semen ejaculate was diluted in Tris Fructose Yolk Glycerol (TFYG) dilutor (FAO-1979), keeping about 50 to 60 millions of spermatozoa per ml. of semen before freezing. The remaining volume of each bull's semen was mixed in a test tube, re-evaluated and extended as above. The diluted semen was filled in 0.5 ml. medium straws and sealed them in polyvinyle alcohol (PVA) powder. The equilibration period was provided as 5 hrs. at 5°C. A "Thermocole Freezing

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CASE REPORTS

JAR 7: 1: 137-139, 1986

Impotentia Couendi And Spastic Paresis In Holstein Friesian Bull

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ABSTRACT

A Holstein-friesian bull '307' aged 4½ years having impotentia couendi was andrologically investigated. The bull was found to be affected with generalized arthritis and accompanied with spastic paresis. A detail case record is presented.

* * *

Clinical andrological investigation of breeding bulls for evaluating them, as regards reproductive soundness periodically, during their reproductive life is most important in order to keep the dairy industry as a profitable enterprise. Many a times, attention of herdsman or semen bank is drawn towards the bulls at a very late stage when sufficient damage has already been caused resulting in lowering of the overall conception rates in the herd or population following N.S. or A.I. Andrological investigation of a H.F. bull carried out at the Department of Gynaecology, GVC, Anand, has been reported.

Case History

A Holstein-friesian bull aged 4½ years, used for A.I. purpose since past 2½ years, was presented for investigation in August, 1981. The bull had strong libido & normal serving behaviour and semen quality as indicated by 2.5 services/pregnancy from the past breeding record. The bull then gradually developed disability to donate semen. Cramps or spastic signs were evident when the bull was suddenly made to stand from recumbency and signs were gradually aggreviated to the extent that the bull could not mount inspite of strong desire and froathy salivation which was observed at every semen collection attempts.

Clinical Andrological Examination

The physical condition of the animal was rather good. The body temperature, respiration and pulse were normal. The animal showed arched back and was unable to bear weight on hind limbs especially right hind limb which was found abducted (Fig). Spastic signs or stretches were not observed when the bull was lying down but evident on standing. The bull was having strong serving ability but serving behaviour was altered. When ever the bull was taken for semen collection, it started shivering specially on hind quarters and back with froathiness and could mount & donate semen with great difficulties during early stage which later on stopped completely due to inability of bull to copulate. Two successive ejaculates were collected at twice a week interval before complete recumbency. The average values were as follows:

Ejaculate volume: 8.00 ml
Color & Consistency: Medium thick creamy.
PH : 7.4 (alkaline).
Mass motility: Nil.
Live sperm percent: 72

Dead sperm percent: 28
Abnormal sperm with maximum head and tail abnormalities: 32%
Sperm concentration: 1800×10^6/ml.

On palpation, the consistency of left testicle was found to be softer than the right, however, the size of both testes was normal. The tubular genitalia and accessary sex glands palpated per-rectally showed no abnormality.

Radiological examination of both lower hind limbs revealed arthritis of fetlock and pastern joints. The joint space was found obliterated.

Cultural examination of joint fluid showed growth of gram positive cocci and proteus organisms but no pyogenic organisms were isolated. Haematological values were almost normal.

The condition of the bull did not respond to the treatment. Later, the condition gradually aggreviated resulting into death of the animal in October, 1981.

Post-Mortem and Histo-Pathological Examinations

On post-mortem examination, the most significant findings observed were: no gross abnormalities of testicles; white spotted kidneys with lesions of infarction; congested, oedematous and haemorrhagic lungs; early autolytic changes in liver and spleen and pin point linear haemorrhage in heart.

On histo-pathological examinations, the testicles were found to be normal. The lymphnodes and lungs were extensively congested. The heart showed severe congestion, focal haemorrhages and infiltration of inflammatory cells. The kidneys revealed same findings as that of heart but in addition, areas of haemorrhage were evident. The liver parenchyma showed degenerative changes,

besides fatty and necrotic changes and accumulation of exudate.

Thus, post-mortem and histo-pathological findings were suggestive of the death due to pyaemia and pyaemic nephritis as a complication of arthritis accompanied by spastic paresis.

Discussion

In spastic syndrome, crampiness or stretches in bull, severe attacks may interfere with or prevent copulation due to prolonged spasms of the skeletal muscles of the rear limbs and back. Spastic syndrome has been observed in all breeds of cattle but most commonly in the Holstein friesian and Guernsey breeds and is probably inherited as a single recessive factor with incomplete penetrance (Roberts, 1965 and Becker

et al., 1966). The condition is seen most often in bulls over 3 years of age and is often associated with arthritis or painful lesions of the rear limbs (Roberts, 1971). The later author has also reported a similar case of spastic paresis in an eight years old Holstein bull. These reports are in close agreement with the present findings. A progressive lameness resembling laminitis eventually resulting in refusal to stand, as observed in the present case, has been described in related Hereford bulls by Brown *et al.* (1967). However, in the present case hereditary nature of the condition could not be ascertained.

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IJAR 7: 1: 140-141, 1986

Fibro-Adenoma Of Cervix In A Pregnant Non-Descript Cow

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Incidence of neoplasms of bovine genital system is rare and cervical tumours are still rare. Few cases of neoplasms of cervix in Indian cows were reported as fibroma (Kohli and Bishnoi, 1980), fibro-leiomyoma (Sindhaye, 1982), Adeno-carcinoma (Bhownik, 1985), Fibro-adenoma (Derashri *et al.* 1985). Fibroma of cervix in buffaloes are also reported (Sane and Purohit, 1958; Rama Rao and Rajya, 1976 and Sharma *et al*, 1977). Authors present here a case of fibro-adenoma of cervix in a pregnant non-descript cow.

Case history and clinical findings:

A 10 year old non-descript cow with earlier 2 calvings was presented as a case of cervico-vaginal prolapse on 2nd Nov. 1985. The cow was bred 4 months before and thereafter she was not observed in heat.

Externally there were no signs of cervico-vaginal prolapse except recurrent straining. Per-rectal examination revealed that the cervix was hypertrophied and closed, but some out-growth was palpable in the vaginal passage. The cow was pregnant for 4 months and it was right horn pregnancy. Per-vaginal examination revealed a rounded, pedunculated, soft tumourous growth with papillary projections originating from lower part of cervix.

Treatment:

Under the influence of caudal epidural anaesthesia the operation was performed in standing position. Well lubricated left hand was introduced per-vaginally and the ontgrowth was secured and exteriorised. The peduncle was ligated with chromic catgut No. 2 and excised with the help of guarded knife. Surgical wound formed at lower part of external os-cervix was cauterised with 2% copper sulphate solution. Post-operative care was taken by giving injections of oxytetracyclin hydro-chloride @ 4 mg/Kg body weight by intra-muscular route for 5 days. Animal showed un-eventful recovery.

Histo-pathological Examination:

Grossly the tumourous growth was pedunculated, pink in colour, soft in consistency having small papillary projections, weighing 300 gms. The growth was collected and preserved in 10% formal saline. Histo-pathological examination of the tissue revealed glandular acini lined by columnar epithelium. The acini were separated by fibrous tissue strands. At some places the acini were cystic and contained mucin. The tumour was identified as fibro-adenoma. No malignant changes could be noticed.

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IJAR 7: 1: 142-143, 1986

Freemartinism In A Cross Bred Animal

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Infertility is one of the pressing problems facing the farmers owning a dairy farm. One of the uncommon cause of such a condition is the so called freemartinism. A freemartin is an infertile female carried in utero with a male co-twin. Ovarian hypoplasia and segmental aplasia are common abnormalities in such cases but differentiation of ovary into testis is rare and hence reported here.

Case history & clinical Examination:

An year old cross-bred heifer (Kankrej × Jersey) was presented for the study with the history of having born as a twin with male calf which died immediately after birth. The animal was looking female from the external genitalia. On clinical examination, the vagina was found ending in a blind pouch with enlarged clitoris. Per-rectal examination revealed ill-defined genitalia. Cervix and body of the uterus were not palpable. Instead, a very thin band was present. On further examination a bifurcation was felt and cord like two horns of the uterus were palpable. Small nodule suggestive of ovary was felt on the right side but it was absent on the left side.

Gross observations of genitalia:

The animal was culled and genitalia obtained on post-mortem showed a gross appearance of an underdeveloped female genitalia. Vagina was shorter than normal and measured 12 cm. in length. Cervix and body of the uterus were absent and in place 8 cm. long thin band of tissue was present (Fig. 1A). This was bifurcated into left (Fig. 1B) and right (Fig. 1C) tubular horns. The circumference of left horn was 3 cm. while that of right horn was 1.5 cm. Left horn was straight and 7 cm. in

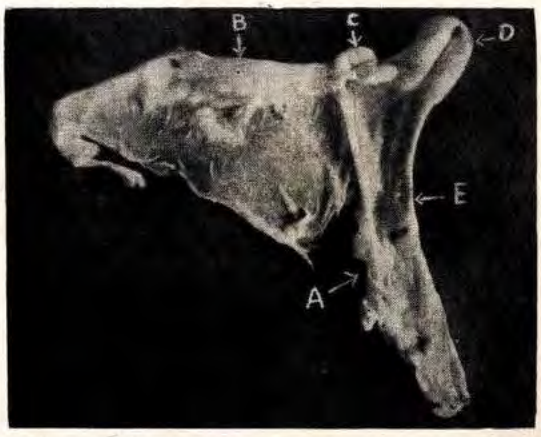

Figure 1.

Internal reproductive organs of a freemartin animal.

A. Cord like structure at the place of uterine body.
B. Left horn of the uterus.
C. Right horn of the uterus.
D. Hypoplastic testis.
E. Epididymis.

length tapering into a structure resembling fallopian tube. There was apparently no ovary like structure on the left side. Right horn was tortuous and 2 cm. in length ending in a smooth hard 'U' shaped structure resembling split ovary (Fig. 1D). Thick cord like structure was extending at the posterior surface of the "ovary-like" structure and measuring 5 cm. long (Fig. 1E) in broad ligament parallel to the thin band of uterine body.

Histo-Pathological Examinations:

Tissue from different parts of the reproductive tract were processed for paraffin sectioning and stained with H. & E. stain. Thin band of tissue present at the site of the uterus revealed longitudinally arranged bands of smooth muscles only, without any ductular structure. Left horn revealed structure of hypoplastic uterus with narrow lumen. Mucosa was lined by columnar cells and contained few scattered uterine glands in the midst of fibrous connective tissue. The right horn was found to show the structure of epididymis with cross sections of few ductus epididymidis widely separated by inter-ductular connective tissue. The 'U' shaped ovary-like structure was found to be hypoplastic testis with single cell layered, atrophic, inactive seminiferous tubules. Large number of interstitial cells were observed in the inter-tubular space. Long cord like structure extending to the posterior surface of the 'U' shaped mass also showed structure resembling epididymis.

Discussion

The condition of freemartimism was described in standard books like Biggers and McFeely, 1966 and Jubb and Kennedy, 1970. The degree of ovarian abnormality in freemartinism varies from simple hypoplasia to complete differentiation into testis.

According to Jubb and Kennedy (loc. cit), the degree of transformation in the male direction probably is related to the stage of development at which anastomosis of the placental blood vessels occurs. During the present study, total replacement of the ovarian tissue by testis indicates the early fusion of the placental circulations. In the present case, tubular genitalia of paramesonephric duct origin varied from a cord like structure without lumina at the place of uterine body, to underdeveloped left uterine horn with lumina and endometrial glands, as observed by Satar (1977). Communication with vagina is always absent, as observed in the present case, no matter how well developed the uterus may be (Jubb and Kennedy, *loc. cit*).

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Hydro-Allantois In A She Buffalo Caused Due To Dropsy And Anasarca Of The Fetus: A Case Report

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ABSTRACT

A case of hydroallantois, fetal ascites, anasarca, oedema of fetal membranes and abnormal adventitious placental unions were reported and probable causes discussed.

* * *

Reported incidence of hydroallantois in black cattle are not many even though study of its occurrence is of great interest, particularly in India where the buffalo population is relatively high. Arthur (1957) has established that the excess of fluid is usually in the allantoic sac and hence should be referred as hydroallantois. A fetus with dropsy and anasarca and born to a she buffalo with hydro-allantois is described in the present communication.

Case History

A cross bred murrah she buffalo aged 6 years, had three previous normal calvings and with a gestation period of 286 days was presented with the history that the abdomen is highly distended and the animal is finding it difficult to lie down as well as to get up.

Clinical Examination and Treatment

The general condition was fair. Even though the animal was partially off feed it had raging thirst. The udder was moderately developed and respiration laboured. By rectal examination fremitus was felt and fetus could not be palpated because of the high fluid content in the uterus. To alleviate the distress, symptomatic treatment was given. The condition slowly deteriorated day after day and on the 292nd day 2 ml of Vetlog (Triamcinolone acetonide-Sarabhai) was given intramuscular. On the 294th day morning the animal developed severe straining even though calving did not take place.

Per-vaginal examination revealed dilated os-uterus with intact fetal bag. The bag was ruptured and the calf was in transverse presentation. The volume of amniotic fluid expelled was approximately three litres and that of allantoic fluid twenty litres. After correcting the fetus to posterior presentation, it was removed by traction. The retained placenta was removed manually. The membranes were found oedematous and many abnormal adventitious placental unions were noticed. Two Nitrofurazone and urea bolus (Furea-Eskay lab) were deposited inside the uterus and repeated after 48 hrs. The animal recovered without further complications.

Examination of the Fetus

It was *still born*, female, *premature* and weighed 49 kgs. The fore head was slightly bulged out with slight malocclusion of the mouth and prognathism of the mandible. The fetus had ascites and there was oedema of the shoulder, arm and fore arm. Lumpy coalacing

masses were noticed at the wither and neck region (Fig.). The abdomen contained 10 litres of slightly straw coloured fluid. The kidneys and liver were oedematous and no other appreciable changes were noticed in other organs.

The lumpy masses sent for histopathological examination were confirmed as oedematous muscular tissue.

Discussion

Most instances of dropsy of fetal sacs of cattle are seen in the last month of gestation and the causes even though not fully known but postulated by Arthur (1957) are placental dysfunction consequent up on incompatability of mother and fetus, less number of functioning cotyledons, and compensatory accessory caruncular development in the pregnant horn and the non-pregnant horn not participating in placental formations, bearing of twins and fetal mal-formations. The excessive accumulation of fluid is obstructive in origin (Jubb and Kennedy, 1963) and in most cases the pathogenesis remain hypothetical. According to them fetal malformations like dropsy and anasarca are causes of hydro-allantois in the dam. Two cases of fetal dystocia due to oedematous muscular masses in Murrah she buffaloes were reported by Sathyasastry (1974) and a case of hydroamnion, fetal ascites and oedema of fetal membranes was placed on record by Sastry *et al.* (1975). Where as in this particular case there was hydroallantois, oedema of the fetal membranes with adventitious placental unions, fetal ascites and anasarca.

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FARM NEWS

Estimation Of Inter-Calving Period In Surti Buffaloes Under Field Conditions (Rural Areas) In Operational Area Of Amul

R.V.K. Jogi, M.C.S. Nair, M.R. Subnis

Amul Research and Development Association, Anand.

The inter-calving period in milch animals is a most important single economical factor influencing the cost of milk production. In Western countries where dairy industries are on sound-footings and selection and culling in dairy cattle are strictly exercised, the limit of inter-calving period is maintained at optimum economically advocated range of 12-13 months. Barring a few exceptional cases, in India where dairying is subsidiary to agriculture and culling of sub-fertile and aged animals is taboo in majority of agriculturist community, the pace between two calving periods is found to be too high. Although scientific literature regarding estimation of inter-calving period of various indigenous breeds on limited number of animals are available from well organised private, govt. and research institutions, the information of this trait on rural milch herd are scanty and are not supported with proper scientific data. In the present text, an attempt has been made to estimate the intercalving period in surti buffaloes being maintained in villages of Kaira district.

Infrastructure available and Mode of operation:

The Kaira district is the only operational area of the well established dairy known as AMUL which is situated in Anand town of Gujarat State. To sustain the interest of Kaira farmers in economical milk production as well as to enhance the milk production per animal, the Amul Research and Development Association (ARDA) has implemented various scientific animal husbandry development programmes and amongst these, Artificial Insemination Programme plays a major role and is placed at the top.

The A.I. work at village level is being done under close supervision of trained Veterinary Officers of ARDA by the village Lay Inseminators, who are also initially trained generally in both liquid and frozen semen insemination technology by the special cell of ARDA. The ARDA has also developed its own indigenous recording system at every A.I. Sub-centre to assess the progress and to study the effect of A.I. programme for its subsequent pregnancy and calving. The main records being maintained at each A.I. centre (sub-centre) are Artificial Insemination Register, Ledger Book, Pregnancy Diagnosis Register, Calving Register, Index Book and Monthly Report Book. Besides this, A.I. Card is given to individual animal owner when the animal is brought for insemination for the first time. Entries in these books are maintained from 1st April to 31st March. The A.I. Register gives the farmerwise (ownerwise) daily account of A.I. performed on his animal while the Ledger Book keeps the individual records of buffalo in respect

TABLE 1. Statement showing yearwise number of buffaloes inseminated and conception rate obtained in Surti breed.

Sr. No.	Year	No. of buff. inseminated	Total no. of insem. performed	No. of pregnancy diagnosis carried out of A.I. served buff.		
				Exam.	Preg.	C.R.
1.	1980-81	1,71,901	2,85,073	1,04,689	56,264	53.74%
2.	1981-82	1,87,108	3,25,886	1,22,624	61,494	50.14%
3.	1982-83	2,00,137	3,53,152	1,27,516	65,134	51.07%
4.	1983-84	1,94,438	3,54,744	1,31,631	65,385	49.67%
5.	1984-85	2,36,963	4,22,876	1,39,587	67,310	48.22%

TABLE 2. Statement showing villagewise pregnancies confirmed and the percentage of cases further successfully followed-up for subsequent pregnancies and calvings

Sr. No.	Name of the village (A.I. Subcentre)	No. of preg. confirmed by A.I. in 1981-82	No. of preg. cases followed up out of 1981-82 cases	No. of cases not traceable after follow-up	No. of cases that could be followed upto subsequent calvings
1.	Popatpura	230	128	51	77
2.	Tranol	237	205	81	124
3.	Bochasan	176	168	59	109
	Total:	543	501	191*	310

* About 38.3% preg. buff. could not be traced up due to transfer, sale, death and confusion in proper identification of animal.

to number of A.I., P.D. results, calving and sex of the calf. Similarly P.D. Register keeps the animalwise detailed account of Pregnancy Diagnosis performed by Union's authorised person. The Calving Report gives the datewise information regarding the number of calvings taking-place in existing animals of the village while Index Book shows the farmer-wise number of animals brought for A.I. in particular year. The monthwise summary of A.I. activities are sent to ARDA's H.O. at Anand for record and analysis.

The recording system indicates (Table 1) that the A.I. programme in Surti buffaloes has been given arouse welcome and well accepted by the farmers of Kaira district.

Methods adopted:

To estimate the inter-calving period in Surti buffaloes, three A.I. Sub-centres covering small (village Popatpura-462 population), medium (village Tranol-768 popu.) and large (village Bochasan 1427 popu.) strength of breedable buffalo population as well as manned with efficient, trained local A.I. worker were ear-marked for the study. From the recorded A.I. data of these Centres, the buffaloes which were confirmed as pregnant in the year 1981-82 were properly traced for their calvings then and subsquent pregnancy and calving in year 1982-83 and 1983-84. The number of buffaloes which were confirmed as pregnant by A.I. and the number which could be followed for

TABLE 3. Statement showing villagewise frequency of calving interval and its percentage attained in Surti buffaloes

Sr. No.	Inter-calving period	Popatpura		Tranol		Bochasan		Over-all	
		Nos.	% to the total	Nos.	% to the total	Nos.	% to the total	Nos.	% to the total
a)	Below 13 months	21	19.3	35	28.22	11	14.3	67	21.61
b)	Above 13 months but less than 15 months	23	21.1	26	20.96	18	23.3	67	21.61
c)	Above 15 months but less than 17 months	12	11.0	13	10.48	8	10.4	33	10.64
d)	Above 17 months but less than 19 months	7	6.4	6	4.83	7	9.1	20	6.45
e)	Above 19 months but less than 21 months	2	1.8	6	4.83	5	6.5	13	4.19
f)	Above 21 months but less than 23 months	17	15.6	15	12.01	3	3.9	35	11.29
g)	Above 23 months but less than 25 months	4	3.7	5	4.03	6	7.8	15	4.83
h)	Above 25 months	4	3.7	8	6.45	4	5.2	16	5.16
i)	Not calved	19	17.4	10	8.06	15	19.5	44	14.19
	Total:	109	100%	124	100%	77	100%	310	100%

TABLE 4. Statement showing villagewise av. inter-calving period in Surti buffaloes

Sr. No.	Name of the village A.I. Sub-centre	Total no. of observation	Inter-calving period	
			Months	Days
1.	Popatpura	62	17	4
2.	Tranol	114	16	16
3.	Bochasan	90	16	25.4
	Over-all	266	16	23

subsequent calvings are entered in table No. 2.

The inter-calving period of 310 buffaloes worked out separately for three A.I. Sub-centres are further split into 7 categories which are mentioned in table No. 3.

Observation:

The study of the table No. 3 indicates that the inter-calving period of 43.2% buffaloes were found below 15 months while 21.3% buffaloes showed their inter-calving period in between 15 to 21 months, 21.3% buffaloes showed their

inter-calving period above 21 months. While the remaining 14.2% buffaloes did not calve (empty) even after 25 months of their respective calvings. Excluding the percentage of buffaloes which remained uncalved, the over-all inter-calving period was estimated for these three sub-centres which are indicated in table No. 4.

Conclusion:

The over-all inter-calving period of 266 cases was recorded as 16 months 22 days. In the year 1960-61 (April), the on-the-spot survey conducted by School Teachers in the operational area of Amul indicated that inter-calving periods in buffaloes were as high as 18-20 months. The reduction of inter-calving period by 2 to 3 months in the area can be definitely attributed to the A.I. programme implemented by the ARDA. Further, this study also indicates that Surti buffalo bears the potential characteristic of annual calver and leave ample of scope to further implement the A.I. programme more scientifically and efficiently in order to achieve this desired potential inter-calving period in local Surti breed within the range of 13 to 14 months even under field conditions.

Acknowledgement:

The authors are thankful to Secretary, ARDA for permitting the study and publishing the results.

IJAR 7: 1: 150, 1986

THESIS ABSTRACTS

Cryopreservation Of Buffalo Semen Using Cryoprotective Sugars

M.V.Sc. Thesis

Student: G.R. Prakash Guide: B. Munilal Dubey

Dept. of Gynaecology & Obstetrics, Veterinary College,
Hebbal, Bangalore-24.

The cryoprotective properties of sucrose-mannose, lactose-fructose in freezing of Surti buffalo semen were investigated using 0.5 ml French straws. Tris extender was used as control which contained 6 per cent glycerol, 20 per cent egg yolk and 1000 IU of Penicillin and 1 mg of Streptomycin per ml. The experimental extenders contained same quantity of egg yolk and antibiotics but without glycerol or buffer.

Sucrose-mannose and lactose-fructose either alone or in respective combinations at 225 nM/lit in the extender without glycerol or buffer gave lower initial prefreeze and post-thaw motility. These sugars did not act as cryoprotectants in absence of glycerol and buffering agent during freezing of buffalo semen.

Lactose-fructose at 312 mM/lit in a semen extender containing 0, 1.5, 3 and 6 per cent glycerol without a buffer showed no significant variation in initial and prefreeze motility. These motilities in the experimental extenders were lower than the control. And it was significant at prefreeze motility. As the glycerol level increased from 0 to 3 per cent, the post-thaw motility increased significantly ($p \leq 0.05$), but it was not observed when glycerol level increased from 3 to 6 per cent and it was lower than the control.

Addition of 73 mM/lit of lactose into a modified tris lactose extender with a total osmolarity of 381 mM/lit had the initial and prefreeze motility slightly lesser than the control extender. The post-thaw motility with 3 and 6 per cent glycerol were higher than the control, but it was not significant. Lactose at 73 mM/lit in a modified tris lactose extender is beneficiary in cryopreservation of buffalo semen with 3 per cent or 6 per cent glycerol level.

Sucrose addition at 73 mM/lit into a modified tris sucrose extender with a total osmolarity of 381 mM/lit behaved similar to modified tris lactose extender with reference to initial and prefreeze motility. The post-thaw motility was better in modified tris sucrose extender containing 6 per cent glycerol, but it was not significant. Presence of sucrose at 73 mM/lit in a modified tris sucrose extender with 6 per cent glycerol is beneficiary in cryo-preservation of buffalo semen.

In modified tris extender, lactose and sucrose at 73 mM/lit level act as cryoprotective in complimentary with glycerol during freezing of buffalo semen in French straws.

IJAR 7: 1: 151, 1986

Studies On Certain Electrolytes And Non-Electrolytes Concentration In Uterine Secretions Of Buffaloes During Pro-Oestrus, Oestrus, Dioestrus And Early Pregnancy

M.V. Sc. Thesis

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Department of Gynaecolgy and Obstetrics, Veterinary College, Hebbal, Bangalore-24

The uterine secretions of buffaloes were analysed for the concentration of inorganic phosphorus, calcium, magnesium, sodium, potassium and chloride. Abattoir material was used for the collection of uterine secretions. The conditions investigated were pro-oestrus, oestrus, dioestrus and early pregnancy.

The inorganic phosphorus concentration in uterine secretions was significantly higher ($p<0.01$) in dioestrus than in pro-oestrus and oestrus. In early pregnancy its level was significantly lower in dicestrus ($p<0.01$), pro-oestrus ($p<0.05$) and higher than in oestrus ($p<0.01$).

The calcium concentration was significantly higher in dioestrus than in oestrus ($p<0.01$) and pro-oestrus. In early pregnancy the concentration was higher than oestrus ($p<0.01$) and dioestrus.

The magnesium concentration was significantly higher ($p<0.01$) in dioestrus than in oestrus. In early pregnancy it was significantly lower ($p<0.01$) than in dioestrus and pro-oestrus.

The sodium concentration during dioestrus was higher ($p<0.01$) than in oestrus and pro-oestrus. In early pregnancy it was lower than dioestrus and significantly higher ($p<0.01$) than in oestrus.

The potassium concentration showed cyclical variation during oestrous cycle and was significantly higher in dicestrus ($p<0.01$) than in oestrus. In early pregnancy it was significantly lower ($p<0.01$) than in dioestrus and pro-oestrus.

The chloride concentration was significantly higher ($p<0.01$) in dioestrus than in oestrus. In early pregnancy it was significantly lower ($p<0.05$) than in dicestrus.

During pro-oestrus, oestrus, dioestrus and early pregnancy the respective mean concentrations were 7.11 ± 0.33, 3.11 ± 0.38, 9.51 ± 0.50 and 5.82 ± 0.40 mg per cent of inorganic phosphorus; 16.53 ± 0.82, 10.82 ± 1.05, 18.06 ± 2.55 and 20.33 ± 2.02 mg per cent of calcium; 15.20 ± 1.10, 7.40 ± 0.98, 15.17 ± 0.97 and 8.83 ± 0.87 mg per cent of magnesium; 291.16 ± 17.00, 174.52 ± 17.60, 325.47 ± 28.40 and 285.46 ± 14.63 mg per cent of sodium; 239.90 ± 8.44, 156.56 ± 16.44, 257.43 ± 8.19 and 180.60 ± 8.40 mg per cent of potassium and 429.07 ± 10.85, 363.43 ± 46.49, 530.53 ± 35.94 and 394.86 ± 54.54 mg per cent of chloride.

The mean values were lowest during oestrus and highest in dioestrus. During early pregnancy their mean values were lower than in dicestrus except for calcium. The present investigation suggests that the developing conceptus utilizes these electrolytes from uterine secretions during development.

The role of gonadal hormones on the homeostasis of various investigated biochemical constituents in uterine secretions have been discussed.

IJAR 7: 1: 152, 1986

Studies On Cervico-Vaginal Mucus Physico-Biochemical Properties Of Cross-Bred Cows At Mid-Oestrus For Prediction Of Ovulation And Fertility

M.V.Sc. Thesis

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Department of Gynaecology and Obstetrics, Veterinary College, Hebbal, Bangalore-24.

The cervico-vaginal mucus collected at mid-oestrus from ovulated and anovulated cross bred cows, and from ovulated animals with fertile heat, infertile heat, and heat in repeat breeders was subjected for two physical and eight biochemical analysis.

The percentage of typical fern pattern in ovulatory and anovulatory heat was 75.5 and 24.5 respectively. Further, in 43 ovulated animals having typical fern pattern 37.52, 16.27 and 46.51 percent of animals had fertile heat, infertile heat and heat in repeat breeders respectively.

The spinnability values and hydrogen ion concentration were higher in ovulated than in anovulated cows. In ovulated groups these values were lower in fertile heat than in infertile heat and heat in repeat breeders. The variation between groups was not significant.

The protein concentration was significantly higher ($p<0.01$) in ovulated fertile heat than in anovulated heat. In ovulated animals its concentration was higher in fertile heat than in infertile heat and heat in repeat breeders, but it was not significant.

The concentration of inorganic phosphorus was lower in ovulatory heat than in anovulatory heat. Whereas the concentration of calcium, magnesium, sodium, potassium and chloride was higher in the former than in the latter. The variation in their concentration was not significant between these two groups. In ovulated groups of animals the inoragnic phosphorus, calcium and potassium concentration was higher in fertile heat than in infertile heat and heat in repeat breeders except the potassium concentrtion was marginally higher in repeat breeders. Further, in ovulated groups the magnesium, sodium, and chloride concentration were lower in fertile heat, than in infertile heat in repeat breeders. The variation in their concentration in ovulated animals between groups was not significant.

The concentration of total proteins in cervico-vaginal mucus at mid-oestrus could be used for predicting ovulatory or anovulatory heat, and fertile or infertile heat in cross bred cows. Further, it is hypothesized that the other investigated characters of cervico-vaginal mucus could be used for predicting not only ovulatory or anovulatory heat, but also fertile or infertile heat. However, further investigations are needed to confirm this hypothesis.

IJAR 7: 1: 153:163, 1986

ISSAR NEWS

item 1

A.

THE FIRST ASIAN CONGRESS ON ANIMAL REPRODUCTION HELD AT BOMBAY ON 11, 12 & 13 DECEMBER 1985....SECRETARIES REPORT

The First Asian Congress on Animal Reproduction was organised by The Indian Society for the Study of Animal Reproduction in association with the Indian Council of Agricultural Research, New Delhi; Konkan Krishi Vidyapeeth, Dapoli; the SCI-TECH centre, Bombay and the Bombay Gorakshak Mandali, Bombay at the new Aarey complex of the Bombay Veterinary College on 11th, 12th and 13th December, 1985. The congress was inaugurated by His Excellency, the then Governor of Maharashtra, Shri K. Prabhakara Rao.

There were delegates from abroad including Dr. M.R. Jainudeen (Malaysia), Dr. M. Fathalla (Iraq), Dr. R.A. Ranasinghe, Dr. A.R. Mohamad and Dr. Mrs. H.M.S.P. Herath (Shri Lanka) and Dr. Meher Singh — F.A.O. Over 300 delegate scientists from various Agricultural Universities, State Veterinary and Animal Husbandry departments, Dairy development departments, Milk Federations, Pharmaceutical companies, Insurance companies, Goshalas and Panjrapoles, participated.

At the inaugural function of the Congress on 11th December 1985, Dr. P.V. Salvi, Vice Chancellor of the Konkan Krishi Vidyapeeth, Dapoli welcomed the guest and Delegates. He appreciated the efforts of ISSAR in bringing Asian and Indian scientists on common forum to discuss problems of mutual interest in Animal Reproduction. He said that the Asian Congress has a special significance since this is held during the centenary year of the Bombay Veterinary College.

Messages of good wishes were received on the eve of the Congress including those of the Prime Minister, Central and State Government Ministers and other dignitaries. These were read out to the audience by Dr. B. C. Dave, Co-ordinating Director of Research and Superintendent of Farms, B.G.M.

In token of our love and appreciation, floral tributes were offered to the Chief Guest and other dignitaries by Dr. C.R. Sane, Patron of ISSAR and Dr. S.B. Kodagali, Editor of the Indian Journal of Animal Reproduction.

During his speech, Dr. B.R. Deshpande, President — ISSAR laid stress on International Co-operation and co-ordination of the scientific research findings in Animal Reproduction in enhancing reproductive efficiency of farm animals thereby augumenting milk production.

The ISSAR Fellowships were conferred by the Chief Guest H.E., the Governor of Maharashtra, on two scientists viz. Dr. R.M. Acharya, Deputy Director General, I.C.A.R. New Delhi and Dr. A. Rama Mohana Rao, Dean, Post graduate studies,

Andhra Pradesh Agricultural University, Hydrabad. Dr. D.P. Velhankar, Hon. Secretary, ISSAR read out the citation of these two scientists highlighting their meritorious contribution to the field of Animal Reproduction.

The Nils Lagerlof Memorial Awards were presented at the hands of the Chief Guest to four scientists for their best publication as detailed below:—

Year	Author	Title of article	Published
1983	Dr. J.C. Dutta Dr. Y.G. Dugwekar	Serum alkaline phosphatase and lactic acid dehydrogenase activity in buffaloes with retained placenta.	Indian J. of Anim. Reprod. *3*:1, 1-4.
1984	Dr. B.S. Prakash Dr. M.L. Madan	Radio-immuno-assay of corticol in peripheral blood plasma of buffaloes post-partum.	Theriogenology, *22*: 3,296.

Dr. A.S. Kaikini, Vice President, ISSAR, read out the citation and briefed the audience regarding salient features of their findings.

Dr. R.M. Acharya, Deputy Director General, I.C.A.R. commended the efforts of ISSAR in promoting the cause of Animal Reproduction and called upon the scientists to intensify research on Physiology of Reproduction in order to qualify for the embryo transfer technique and deep freezing of semen.

H.E., The Governor of Maharashtra, in his inaugural speech implored the scientists to pay more attention on the health cover of animals and appealed that efforts should be concentrated on reproduction of quality animals.

Dr. M.L. Madan, Joint Secretary ISSAR, proposed a vote of thanks and the Inaugural function concluded with the National Anthem.

On the same day after noon and following two days, there were 10 technical sessions during which over 165 technical papers were presented and discussed. In order to accomodate such a huge number of papers, two simultaneous sessions were run in two separate halls concurrently.

In addition to above there were 4 Guest Lectures as detailed below:

Sr. No.	Guest Speaker	Topic
1	Dr. R.M. Acharya Deputy Director General I. C. A. R. New Delhi.	Sire seletion of Dairy Cattle breeding.
2	Dr. H.C. Pant Professor of Gynaecology College of Veterinary Science MATHURA (U.P.)	Hormones and Hormcnal Therapy in Bovine Reproductive disorders.
3	Dr. M.L. Madan Prof. & Head of Production Physiology National Dairy Res. Institute KARNAL	Super ovulation in Bovines

4	Dr. M.R. Jainudeen Head of Clinical Depts. University of Pertanian MALAYSIA	Reproduction in Swamp Buffaloes

In addition to these, there were two panel discussions on (i) Herd management in relation to fertility and (ii) Progress through years in Reproduction of cattle and Buffaloes as influenced by A.I./Cross breeding.

During the three days of the Congress, film shows on i) Oestrus detection in cattle, ii) Embryo transfer in sheep/cattle, iii) Miracle of life and iv) Embryo transfer in cattle (West Germany) were shown to the delegates. A small exhibition was also organised wherein pharmaceutical companies and the manufacturers of A.I. equipment participated. This evoked a lot of interest. Some of the delegates also visited C.B.F. Kandivali and were happy to note the progress of the Institution.

The technical sessions were followed by the Plenary session which was chaired by Dr. A.S. Kaikini. The recommendations arising out of these are printed elsewhere. The First Asian Congress came to an end with a valedictory address by the Associate Dean, Bombay Veterinary College, on 13th December 1985 at 19.30 hours.

B.

RECOMMENDATIONS OF THE PLENARY SESSION OF THE FIRST ASIAN CONGRESS ON ANIMAL REPRODUCTION HELD AT BOMBAY ON 13-12-85.

1) It was unanimously resolved that a common forum for Asian Countries should be formed for exchange of scientific information in respect of Animal Reproduction. Further there should be free exchange of technology between the countries and that scientists be allowed to visit for mutual benefit.

2) As sufficient experience and expertise is available, it is recommended to establish centres of excellence in the following areas: (i) Andrology (ii) Frozen Semen Technology (iii) Reproductive disorders (iv) Obstetrics (v) Endrocrinology & (vi) Embryo Transfer Technology.

3) With the increased scientific investigations involving immunological and hormonal studies, it was resolved that research in immuno-reproduction and standardization of hormonal assay procedures be intensified for augmenting fertility.

4) It was recommended to intensify research on the methods of heat detection in bovines to enhance reproductive efficiency.

5) More stress should be laid on the research in managemental and nutritional aspects of infertility in farm animals.

6) It was recommended to create facilities for standard reference work on clinical problems encountered in the field. Such facilities will be of immense use to the clinicians and research workers as well.

7) It was reiterated that all bulls utilised for A.I. work should be strictly screened for sexual soundness and sexually transmitted diseases. Further, it is recommended that a separate organisation be created by I.C.A.R. where in the approved bulls meant for

A.I. are registered alongwith their detailed performance data. Such a facility will enhance the utilicty of outstanding sires.

8) Orientation courses for the field staff in respect of deep freezing of semen technology and handling of deep frozen semen be conducted periodically in order to achieve better results of fertility in the field.

9) It was recommended that the I.S.I. should fix standardization in the A.I. Guns, disposable sheath and other A.I. equipment, rubber/latex wares used in A.I. work in consultation with the A.I. experts. Further, it should be made obligatory on the manufacturers of the above to follow the ISI Standards.

10) With indescriminate use of antibiotics in the treatment of infectious infertility, increasing cases of drug resistance are being encountered. It was suggested that use of Iodine preparations especially for intra-uterine medication be resorted to.

11) It was recommended that more attention should be paid for udder health control programme in order to achieve higher milk yield.

12) The I.C.A.R. has prepared and recommended model syllabus in the subject of Animal Reproduction (Obstetrics, Gynaecology, Andrology and A.I.). In those Agricultural Universities where the model syllabus is not followed, they be requested to implement the same in toto.

I.S.S.A.R. gratefully acknowledges the financial assistance sanctioned by the I.C.A.R. New Delhi for holding the First Asian Congress on Animal Reprodnction in December 1985.

(Dr. D.P. Velhankar)
Organising Secretary
First Asian Congress on
Animal Reproduction.

2.

NOTIFICATION

NILS LAGERLOF MEMORIAL AWARD

The Indian Society for the study of Animal Reproduction is pleased to invite Research/clinical articles on the subject of Animal Reproduction published by Indian authors in any of the journals during January to December 1985 for consideration of the "Nils Lagerlof Memorial Award" for the year 1985.

Four copies of the reprints of the articles should be sent by the author to Dr. D.P. Velhankar, Hon. Secretary, ISSAR, Veterinary Polyclinic, opp. Nirlon, Western Express Highway. Goregaon East, Bombay—400 065. The articles should reach the Hon. Secretary, ISSAR, latest by 15th September 1986. The award will be presented at the inaugural function of the "Sixth National Congress on Animal Reproduction" to be held at the Assam Agricultural University, Guwahati, Assam on 28th November 1986.

Bombay
Date: 21-7-86

(Dr. D.P. Velhankar)
Hon. Secretary, ISSAR.

3.

NOTIFICATION

The Indian Society for the Study of Animal Reproduction in association with the Assam Agricultural University and the Indian Council of Agricultural Research, New Delhi proposes to hold the "Sixth National Congress on Animal Reproduction" at Guwahati (Assam) from 28th to 30th November 1986.

Research/Clinical papers may please be forwarded to Dr. C.K. Rajkonwar, Organising Secretary, Sixth National Congress on Animal Reproduction, College of Veterinary Science, Assam Agricultural University, Khanapara Campus, GUWAHATI, Assam, 781 022. Inquiries with regard to accomodation, reservation, delegate fees, climate etc. can be made to the Organising Secretary.

Bombay
Date: 21-7-86

(Dr. D.P. Velhankar)
Hon. Secretary,
ISSAR

4.

ANNOUNCEMENT FOR THE SIXTH NATIONAL CONGRESS ON ANIMAL REPRODUCTION AT GUWAHATI-ASSAM

On behalf of the Indian Society for the study of Animal Reproduction in association with the Indian Council of Agricultural Research, New Delhi, Assam Agrioultural University and the State A.H. & Veterinary Department, Assam, it is proposed to organise the "Sixth National Congress on Animal Reproduction at Guwahati (Assam) from 28th to 30th November 1986.

The last date of receipt of the abstracts of the scientific papers is *31st August 1986* and for submission of full text of paper in duplicate is 30th September 1986. The Delegate fee is Rs. 150/-

For further details please contact Dr. C.K. Rajkonwar, Organising Secretary, Sixth National Congress on Animal Reproduction, Professor & Head, Department of Gynaecology, Obstetrics and Artificial Insemination, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati- 781 022

Dr. D.P. VELHANKAR
Hon. Secretary,
I.S.S.A.R.

Dr. C.K. Rajkonwar
Organising Secretary
6th National Congress on
Animal Reproduction.

5.

11th INTERNATIONAL CONGRESS ON ANIMAL REPRODUCTION AND ARTIFICIAL INSEMINATION

To be held at University College Dublin, from June 26th to July 1st 1988. Plenary session topics to include Neuro-endocrine control of reproduction, gene transfer/embryo

manipulation in animal production, establishment of pregnancy. In addition there will be several symposia relating to many areas of animal reproduction.

Further information is available from: Dr. Maurice Boland, U.C.D., Lyons Estate, Newcastle, Co. Dublin. Ireland.

6.

Endocrine Society of India is holding next mid term meeting at the Institute for Research in Reproduction, Bombay from 4th to 8th November, 1986 For full details contact. Dr. Chander P. Puri Organizing Secretary, Institute For Research in Reproduction, Jehangir Merwanji Street Parel, Bombay — 400 012

7.

Dr. M.L. Madan, Joint Secretary Indian Society for the Study of Animal Reproduction and Head, Animal Physiology Division, NDRI, Karnal attended the First World Buffalo Congress held in Cairo, Egypt in December, 1985. The Congress was held under the auspices of Egyptian Veterinary Association for /Buffalo Development, Guiza Egypt & University of Florida Gaines-Ville, U.S.A. Dr. Madan was awarded the Science Pioneer Prize for his valuable Scientific contributions. He was also conferred an Honorary Fellow of the Egyptian Veterinary Association. Members by of ISSAR are proud of his achievements. His participation was sponsored by the International Development Research Centre, Canada.

Dr. M.L. Madan receiving the Science Pioneer Prize at the Egypt.

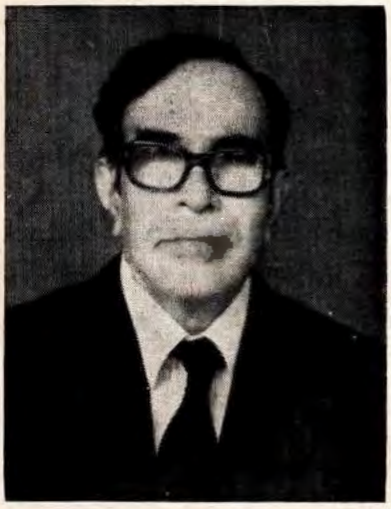
8.

"DOCTOR OF SCIENCE"

(honaris Cause)

to

Dr. C. R. Sane



Dr. C. R. Sane, Patron and ex-President of ISSAR; Ex-Principal, Bombay Veterinary College, Dean, Faculty of Technology, University of Bombay, and currently the Director of Research and Veterinary Advisor, B.G.M. was felicitated and awarded the honorary degree of "Doctor of Science, (honaris-cause) for his meritorious contribution to Veterinary Education, Research and Extension, specially in the field of Animal Reproduction, by His Excellency, Dr. Shankar Dayal Sharma, the Governor of Maharashtra and Chancellor of the Agricultural Universities at the twelth Convocation of the Konkan Agricultural University, Dapoli, on 25th May, 1986.

Dr. Sane is recipient of 'Nils Lagerloff Memorial Award' (ISSAR-1981) and the "Best Veterinary Academician Award" (1985).

He is the Chief Editor of the Text-book on "Reproduction in Farm Animals (Theriogenology)" and Member of the Editorial Board of the "Indian Journal of Animal Reproduction."

Dr. Sane gets the creidt for having done pioneering work in implementing 'Breeding by Artificial Insemination in Cattle and Sheep' in the State of Maharashtra. He was vitally involved in implementing Cross-breeding of the indigenous Gir cattle with exotic Holstein and Jerscy at C.B. Farms, Kandivli and in producing cross-bred cow Mamata-II which gave the highest yield of 53.1 litres of milk at her peak in the third Lactation and won the 'Gopal Ratna' Award. (1981).

As Director of Research and Veterinary Advisor, B.G.M. Dr. Sane has given spot guidance to four students for the degree of Ph.D. and twenty-six stdents for the degree of M.V.Sc.

Dr. Sane also gets the credit for having placed on record for the first time in India, "Infertility due to Copper-Deficiency", and "Presence of Vibrio-fetus infection in Cattle".

Dr. Sane has 112 research/clinical publications to his credit, specially in the subject of Animal Reproduction.

Dr. C.R. Sane,

G.B.V.C. (Bom); F.R.V.C.S. (Sweden); D.Sc. (Hon)

Director, Institute for Research and Development of Dairy Cattle, B.G.M.

Veterinary Advisor, Sci Tech Centre, Bombay.

Patron and Ex-President, Indian Society for the Study of Animal Reproduction.

Vice-Chairman, Indian Association of Fertility and Sterility.

Member, Indian Society for the Study of Reproduction & Endocrinology.

Member, Indian Veterinary Medical Association.

Member, Editiorial Board, Indian Journal of Animal Reproduction (I.S.S.A.R.)

Formerly, Professor of Obstetrics and Gynaecology, Principal, Bombay Veterinary College and Dean, Faculty of Technology, University of Bombay.

Recipient: NILS LAGERLOF MEMORIAL AWARD-1981 (ISSAR).
BEST VETERINARY ACADEMICIAN AWARD, 1985.

Chief Editor,
Text Book on Reproduction in Farm Animals
(Theriogenology).

Obituary

Biodata of Dr. Guru Bachan Singh

The members of ISSAR will be shocked to learn the untimely passing away on 24th February, 1986 at Chandigarh of late Dr. G.B. Singh who was Professor Emeritus, Department of Obstetrics and Gynaecology, College of Veterinary Science, P.A.U., Ludhiana and founder member of ISSAR. He was responsible for holding the First All India Symposium on Animal Reproduction at Ludhiana in 1977. He was awarded fellowship of ISSAR and D.Sc. from O.U.A. & T., Bhubaneswar.

Dr. G.B. Singh was of 80 years and obtained M.R.C.V.S. from London in 1939. He was Deputy Director of Veterinary Services, Bihar, 1940; Professor of Surgery, Bihar Veterinary College, Patna and Director of Veterinary Services, Orissa. He obtained F.R.V.C.S. in Animal Reproduction at the Royal Veterinary College, Stockholm in 1954. He was FAO Expert in Syria, Somali and Iraq during 1959-76.

In his passing away, the members of ISSAR have lost one of its most distinguished builders. His dedication in the field of Animal Reproduction Education, Research and Extension will long be remembered in India and abroad.

The members of ISSAR share the sorrow and pay their Homage. May his Soul rest in peace.

K. J. KAPADIA & CO.,
CHARTERED ACCOUNTANTS
167/71, DR. VIEGAS STREET,
GAIWADI, KALBADEVI,
BOMBAY — 400 002

Name: THE INDIAN SOCIETY FOR THE STUDY OF ANIMAL REPRODUCTION

Address: Department of Animal Reproduction, Bombay Veterinary College, Parel, Bombay 400 012

INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31ST MARCH- 1985.

EXPENDITURE	AMOUNT	INCOME		AMOUNT	Accounting
To Souvenir Printing	32,412-50	By Donations		1,000-00	Year
„ Lunch, Refreshments	120-00	„ Subscription		1,964-00	1984-85
„ Travelling	300-00	„ Membership Fees		18,069-00	
„ Postage and Telegrams	1,419-70	**By Bank Interest:**			
„ Stationery	719-50	F.D.R. Interest	568-94		
„ Conveyance	357-90	Savings Interest	1,325-20	1,894-14	
„ Bank Charges	130-05	By Advertisements		15,750-00	
„ Audit Fees	502-00	„ Misc Receipts		4-00	
„ Miscellaneous	250-00				
„ Surplus of Income over Expenditure transferred to Balance sheet	2,469-49				
	Rs. 38,681-14			Rs. 38,681-14	

For K.J. KAPADIA & CO.,
Sd/-
(Chartered Accountant.)

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President
ISSAR

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(DR. D. P. VELHANKAR)
Hon. Secretary
ISSAR

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BOMBAY — 400 002

Accounting Year 1984-85

Name: THE INDIAN SOCIETY FOR THE STUDY OF ANIMAL REPRODUCTION

Address: Depart of Animal Reproduction, Bombay Veterinary College, Parel, Bombay — 490 012

BALANCE SHEET AS ON 31ST MARCH, 1985

LIABILITIES		AMOUNT	ASSETS		AMOUNT
TRUST FUND:			BANK BALANCE:		
Balance b/f.	61,306-60		In current Accounts		
			Bombay	47,964-65	
Add: Surplus during the year	2,469-49	63,776-09	Hissar	256-47	48,221-12
			CASH ON HAND:		
			Bombay	97-71	97-71
			FIXED DEPOSIT:		
			with S.B.I.		6,258-26
Nils Lagerl of Memorial Fund	801-00		AMOUNT RECEIVABLE FROM I.C.A.R.:		
			Symposium	5,000-00	
			Journal	5,000-00	10,000-00
		Rs. 64,577-09			Rs. 64,577-09

For K. J. KAPADIA & CO.,

Sd/-
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President
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3 BROTHERS

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