

VOL. 6 No. 2 : DECEMBER 1985



The Indian Journal of Animal Reproduction

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Registration No 533

(SOUVENIR ISSUE)

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Editorial

DEVELOPMENT OF NEW TECHNOLOGIES IN ANIMAL REPRODUCTION*

The cattle industry has successfully adopted artificial insemination almost exclusively, thereby exploiting the potential of available proven sires. The great potential left to exploit the remaining half of the herd (dam) initiated embryo transfer in cattle. Embryo transfer tool is a composite technology. The term embryo transfer has become the appellation for superovulation, embryo recovery, short term in-vitro culture, embryo transfer and cryopreservation of the embryos. Preservation by freezing offers a new form of "biological insurance" and also opportunities in medical research like treating cancer by immuno-therapy, wherein a tumour is removed from a patient for preparing vaccine, frozen and thawed and then later used for therapy. Amongst poultry husbandry also there have been several reproductive technologies such as A.I. and semen preservation (Beltsville Method) that have aided or accelerated the advances made through quantitative genetic technology. An encouraging degree of fertility (50%) has been obtained with frozen turkey semen. Aquaculture in an ancient concept, however its science is far behind that of terrestrial animal husbandry. Aquatic organisms are highly suited for culture because they devote more food-energy to growth than terrestrial animals and produce more protein per unit of water than terrestrial agriculture. In developed countries, aquatic animals are also hormonally induced to ovulate, unlike our farm animals which exhibit low fecundity and yield thousands of eggs at ovulation.

Further, man's desire to control sex of his animals must be as old as his domestication. Many workers have claimed to have separated X and Y bearing spermatozoa and each year that passes seems to bring new claimant but no one has been able to confirm it. However, methods of prenatal diagnosis of sex are available such as sex chromatin and or Y body identification, sex chromosomal analysis, demonstration of H-Y (male) antigen and hormonal assay. In-vitro fertilization, coupled with embryo transfer and normal gestational development has also been reported in rabbit, mouse, rat, cow and human. The vulnerability of recently shed ova and difficulties associated with sperm penetration has prompted research towards achieving early embryonic development of bovine, porcine and hamster oocytes within oviducts of pseudopregnant rabbits—a process referred to as Xenogenous Fertilization. Nuclear transplantation, also known as cloning was primarily designed as a procedure to ascertain whether or not somatic nuclei of developing embryo retain all genetic information found in a zygote nucleus.

Amphibian cloning requires insertion of a totipotent nucleus into an enucleated mature ovum. In mammals the procedure is analogous. Micro-injection of sperm, just the reverse of micro-surgical removal of male pronuclei, is the simple variant of transplanting nuclei, thereby eliminating use of large number of sperm — one sperm per oocyte would be sufficient. Identical twins are natural examples of cloning. Parthenogenesis, a method of selfing, occurs naturally in all vertebrate class except mammals; suppressing meiosis and activating oocyte without involving sperms would be ideal way to produce clones in animals. If successful in milch cattle, this would lead to production of very valuable dairy herd without the need of bulls. Gene transfer occurs naturally during fertilization. Methods are being devised to control it. Recombinant DNA technology, using recently discovered enzymes and the viruses and genes as vector for other genes, now permits introduction of selected genes into a given cell. Recently developed human monoclonal antibodies, isolation or production of interferons, could be invaluable tools in immunodetecting and immunotherapy of tumours.

Lastly, even though, the implementation of new technologies depend on interaction between many factors, beginning with a decision to strive for improvement, research and development to demonstrate feasibility, managerial decisions and fore-sighted planning, the research and development must be a constant on-going process to maximize benefits to society.

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Utility Of Endometrial Biopsy Instrument In Diagnosis Of Fertility In Cows And Buffaloes

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ABSTRACT

An instrument was devised for collection of endometrial biopsy in cows and buffaloes. Biopsy tissues weighing 75 to 150 mg could be obtained with the instrument during insemination period in cows and buffaloes. Detrimental effects of biopsy operation on fertility were not observed in cows and buffaloes.

* * *

Various models of trocar-cannula and jaw type of endometrial biopsy instrument were used in cows and buffaloes (Skjerven, 1956; Mcqueen, 1967; Sinha, 1980). Significance of endometrial biopsy technique in diagnosis of fertility was elucidated by Mcqueen (1968) in cattle and Kenny (1978) in mares. Endometrial biopsy technique was employed to determine functional state of endometrium during estrous cycle (Kenny *et al.*, 1965) and involution period (Veronesi, 1978) in cattle. Scanty information is documented on clinical application of endometrial biopsy as a safest technique in diagnosis of fertility in cattle. Therefore, present study was undertaken to elucidate utility of endometrial biopsy technique in fertility investigations in cows and buffaloes.

Materials and Methods

Present study was carried out on 40 crossbred cows and 40 Murrah buffaloes

maintained under optimum management at Animal Science Farm, Punjab Agricultural University, Ludhiana. Vasectomized bulls were used for detection of estrus in cows and buffaloes and confirmation was made by recto-vaginal examination. Endometrial biopsies were collected with trocar-cannula model of endometrial biopsy instrument (Table 1, Fig. 1) from normal group of animals during insemination period and the post-biopsy conception rate was compared with control group to elucidate effect of biopsy on fertility.

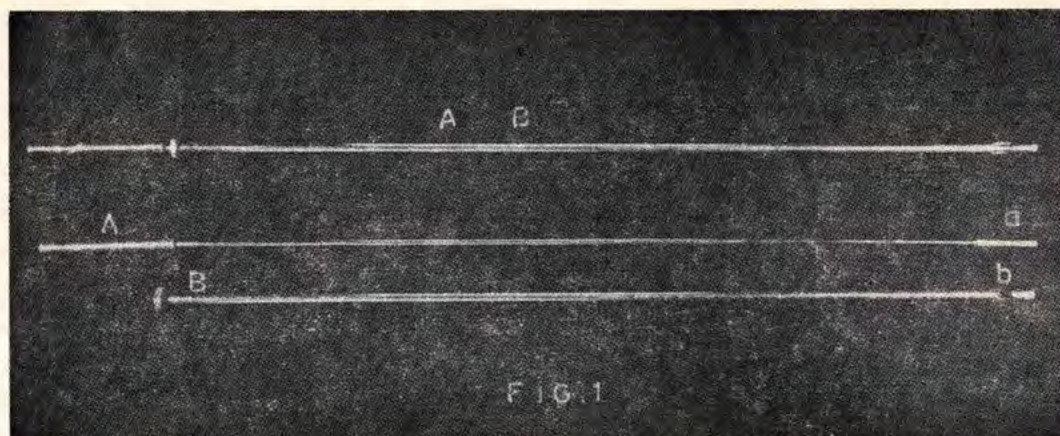
Experimental animals comprised of two groups—

i. *Normal group*—Animals having normal genitalia with regular estrous cycle and conceived within three consecutive inseminations following collection of endometrial biopsy.

ii. *Control group*—Animals which had normal genital organs, regular estrous cycle and conceived within three successive inseminations. These animals were not subjected to biopsy operation.

Results and Discussion

Trocar-cannula model of endometrial biopsy instrument was found to be 100 per cent successful for biopsy collection in cows and buffaloes. Endometrial tissues obtained with the instrument were sufficient for metabolic studies involving biochemical fractionation. Sinha (1980)



LEGENDS FOR FIG. NO. 1
ENDOMETRIAL BIOPSY INSTRUMENT

- AB — Assembled biopsy instrument
- A — Trocar
- a — Cutting edge of Trocar
- B — Cannula
- b — Milled cavity of cannula

also used endometrial biopsy instrument similar to the present model with a guard speculum and collected sufficient sized biopsies. The Nielsen's biopsy instrument was found to be 66.66 per cent effective in collection of endometrial biopsy from cows (Singh, 1979), and was inadequate for biochemical investigations (Mcqueen, 1967).

Mcqueen (1967) designed trocar-

TABLE 1: Specifications of trocar-cannula model
endometrial biopsy instrument

Measurements	Parts of biopsy instrument		
	Trocar	Cannula	Milled cavity
Length (cm)	58.0	50.5	1.2
Diameter (mm)	5.0	6.0	—
Width (cm)	—	—	0.5

cannula type of endometrial biopsy instrument with clockwise and counterclockwise function of trocar whereas present model and instrument used by Sinha (1980) functioned by sliding action of trocar. Cervical trauma was often evidenced with jaw model of biopsy instrument (Skjervén,

TABLE 2: Conception rates in normal and control groups of cows and buffaloes

No. of A.I.	Cows		Buffaloes		Total Animals	
	Normal (n=20)	Control (n=20)	Normal (n=20)	Control (n=20)	Normal (n=40)	Control (n=40)
1st	14 (70.0)	13 (65.0)	12 (60.0)	10 (50.0)	26 (65.0)	23 (57.5)
2nd	5 (25.0)	4 (20.0)	6 (30.0)	8 (40.0)	11 (27.5)	12 (30.0)
3rd	1 (5.0)	3 (15.0)	2 (10.0)	2 (10.0)	3 (7.5)	5 (12.5)
(X ²)	1.15		0.47		0.73	
Value	NS		NS		NS	

Figures in parentheses are percentages
NS = Non-significant

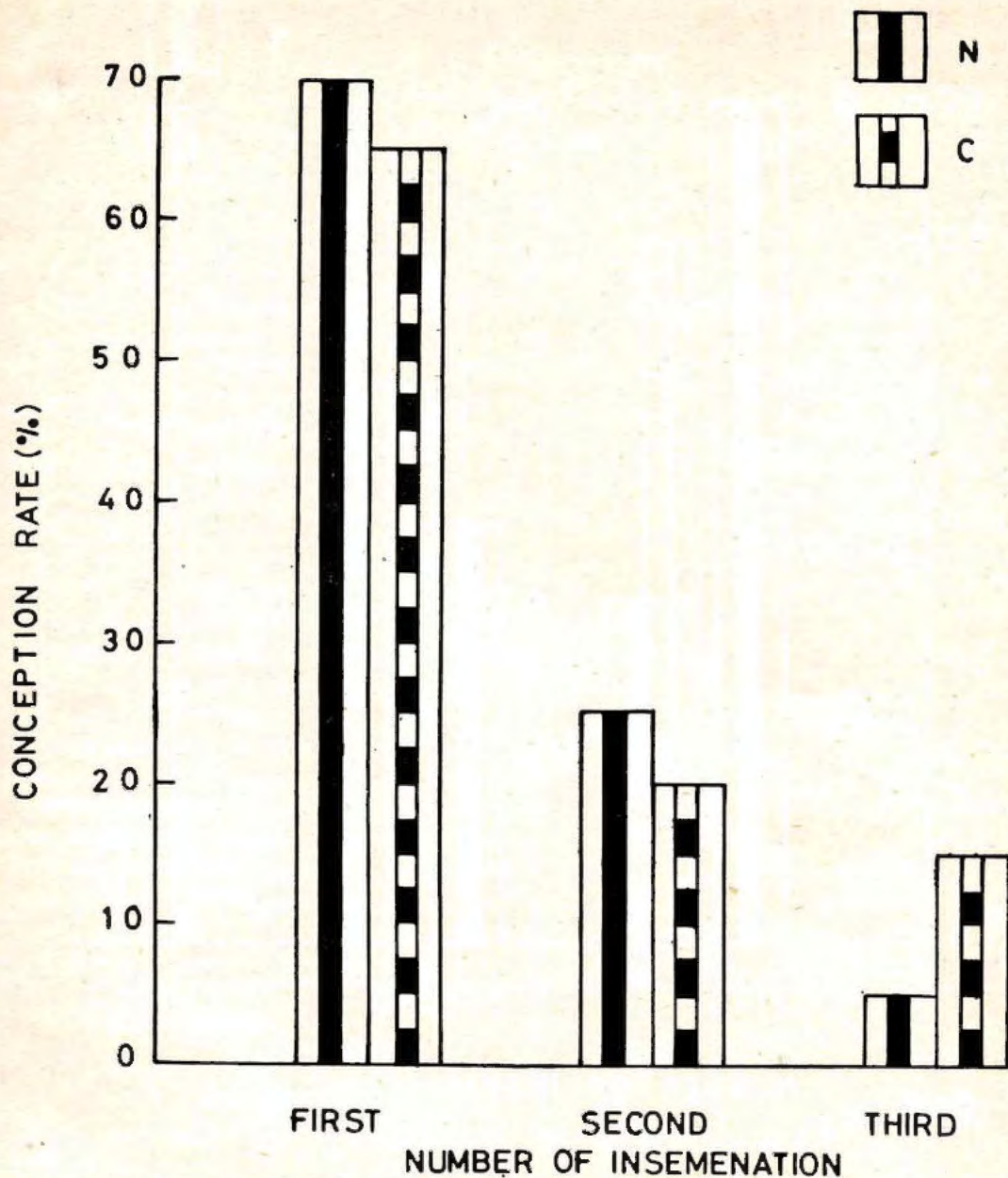


FIG.2. MEAN CONCEPTION RATE IN NORMAL (N) (POST-BIOPSY) AND CONTROL (C) GROUPS OF COWS.

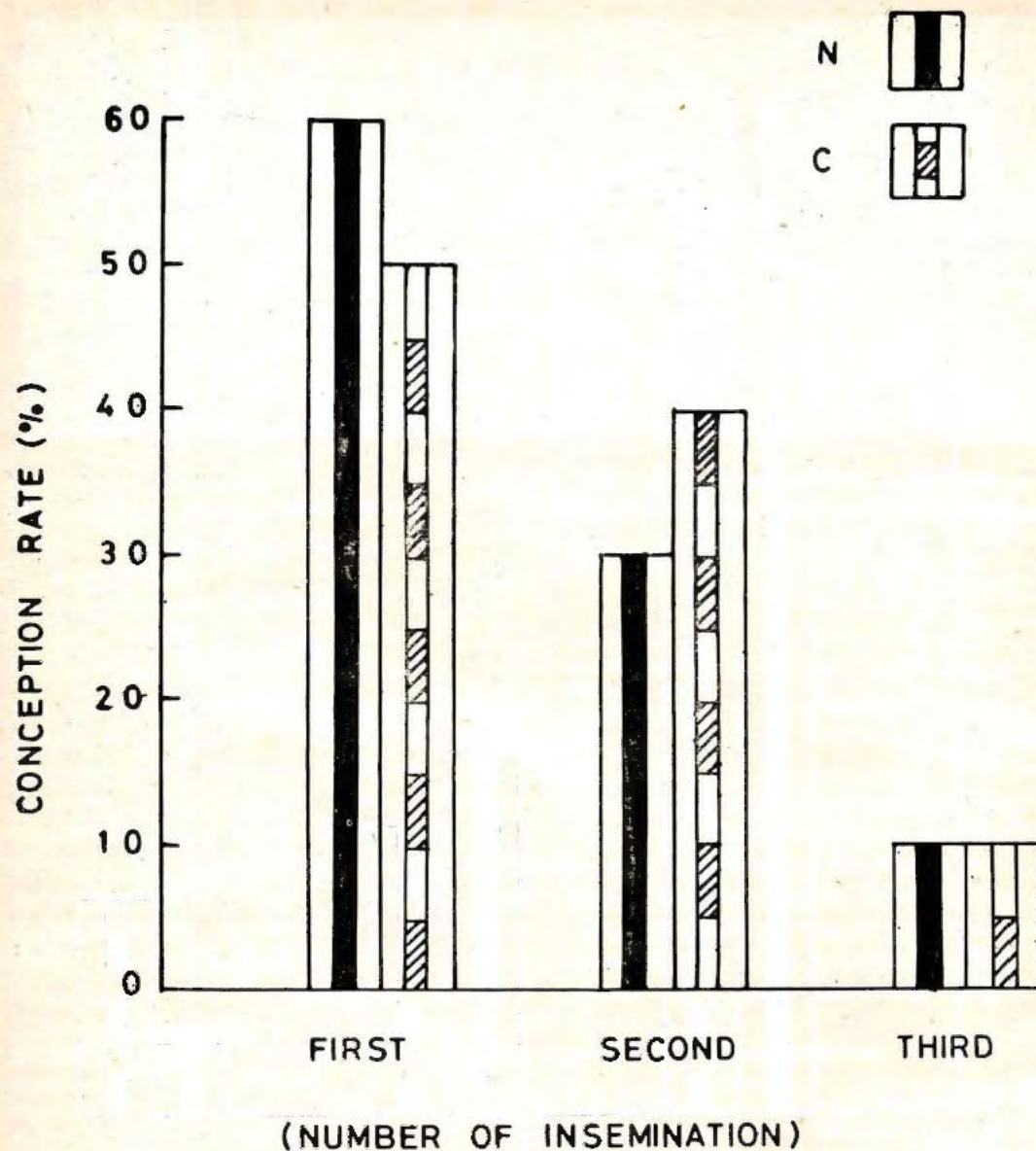


FIG. 3. MEAN CONCEPTION RATE IN NORMAL (N) (POST-BIOPSY) AND CONTROL (C) GROUPS OF BUFFALOES.

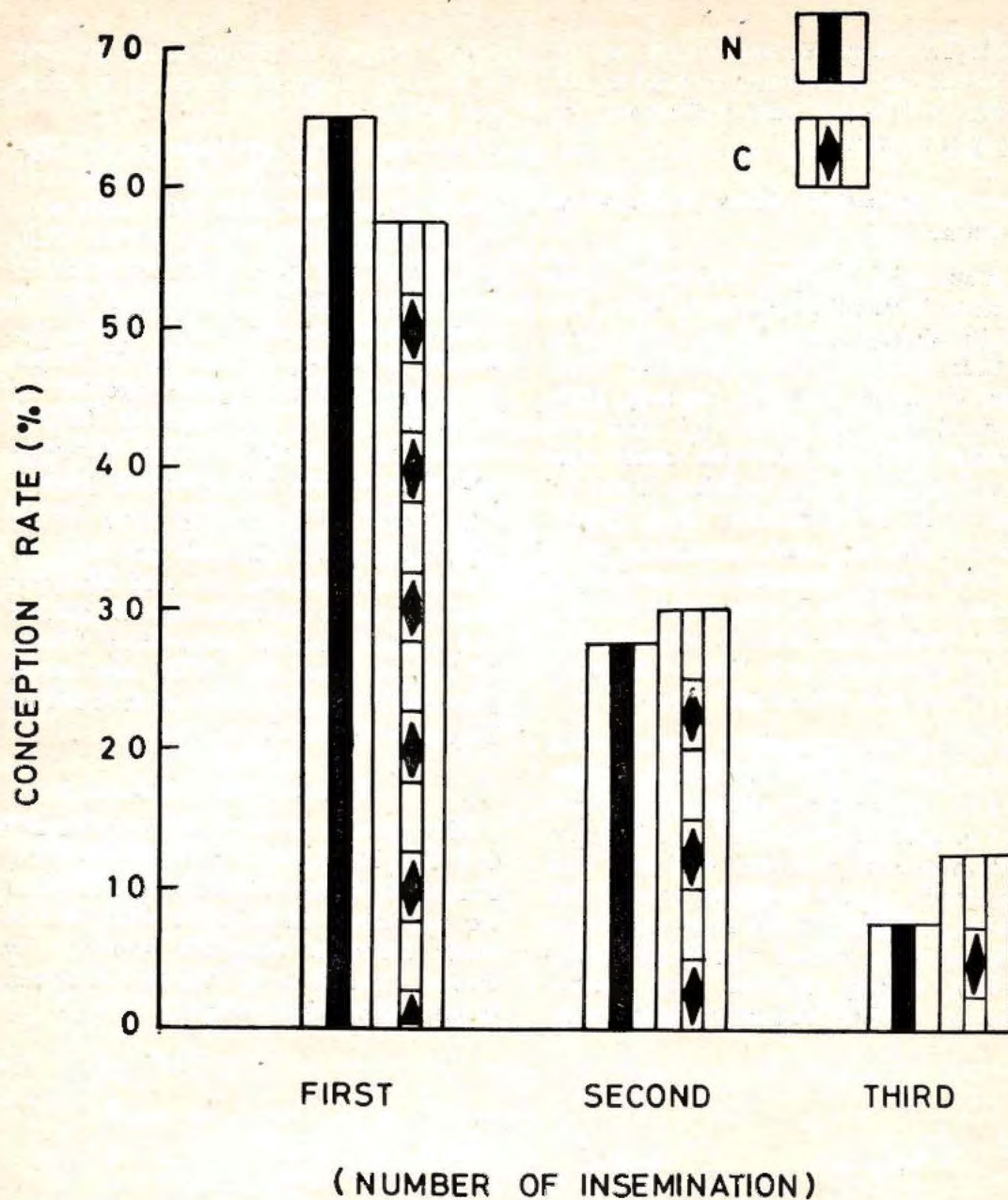


FIG. 4. MEAN CONCEPTION RATE IN NORMAL (N) (POST-BIOPSY) AND CONTROL (C) GROUPS OF ANIMALS (COWS+BUFFALOES).

1956). The tapered and round tip of the present model avoided cervical injury during instillation and withdrawal of biopsy instrument.

Effect of endometrial biopsy technique on conception:

Endometrial biopsy technique was found to have no detrimental effect on fertility since non-significant differences in first insemination conception rates were observed between normal (70.0 per cent) and control (65.0%) cows and between normal (60.0 per cent) and control (50.0 per cent) buffaloes (Table 2; Figs. 2 & 3). Similarly, Brus (1954) reported 75 per cent conception rates in cows with immediate post-biopsy insemination. The recorded first insemination conception rates in normal cows are in agreement with that of Skjerven (1956) in cows (68.85 per cent).

The second and third insemination conception rates were 25.0 and 5.0 per cent in normal cows, 20.0 and 15.0 per cent in control cows, 30.0 and 10.0 per cent in normal buffaloes 40.0 and 10.0 per cent in control buffaloes, respectively (Table 2; Figs. 2 & 3). Non-significant differences in second and third inseminations among normal and control animals further indicated that endo-

metrial biopsy technique could be used as a safest diagnostic method in cows and buffaloes.

The overall first insemination conception rates in normal (65.0%) and control (57.50%) animals are in accordance with that of Sinha (1980) who reported 63.38 and 60.25 per cent first insemination conception rates in normal and control animals, respectively (Table 2, Fig. 4). Similarly, 66.66 per cent and 58.33 per cent conception rates were reported by McQueen (1967) and Skjerven (1956), respectively in cows subjected to biopsy operation during post-breeding period.

The over all conception rates of the herd were 27.5 and 30.0 per cent on second insemination, 7.5, and 12.5 per cent on third insemination in normal and control animals, respectively (Table 2; Fig. 4). Veronesi (1978) also could not observe adverse effect of endometrial biopsy on fertility in cows.

Acknowledgement

The authors would like to thank Dr. S. S. Gill, Head, Deptt. of Animal Science, Punjab Agricultural University, Ludhiana for providing the animals used in this study. Financial assistance awarded by Indian Council of Agricultural Research is gratefully acknowledged.

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Plasma Progesterone Profile In Cystic Ovarian Disease Of Dairy Cows

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ABSTRACT

Jugular blood plasma progesterone concentration was measured in 14 cows and 3 heifers with cystic ovaries. Only one cow showed a low plasma progesterone concentration (<1 ng/ml) throughout the period of study, that is 65 days. In 11 cows with follicular cysts, the period of occurrence of low plasma progesterone profile varied from 8 to 57 days. In these cows, at other times, the progesterone concentration was more than 1 ng/ml. In one cow and three heifers with luteal cysts, there was a high plasma progesterone concentration. The life span of cysts ranged from 23 to 58 days. Subsequent to spontaneous recovery from cysts, conception occurred in 4 cows, and regular oestrous cycles with a repeat breeder syndrome occurred in 3 cows and 2 heifers. In one heifer and seven cows, the cysts recurred.

* * *

The cystic ovarian disease is associated with infertility in dairy cows. The cystic ovarian disease could be classified into two groups; 1. follicular cysts and 2. luteal cysts (Dawson, 1957; Kesler & Garverick, 1982). Plasma progesterone concentration has been measured over a short period in cows with naturally occurring cystic ovaries (Glencross & Munro, 1974; Kesler, Garverick, Caudle, Elmore, Youngquist & Bierschwal, 1980). In the present investigation,

plasma progesterone was measured in dairy cows with or without spontaneous recovery from naturally occurring cystic ovarian disease.

Materials and Methods

Eighteen dairy cows (four control and fourteen cystic ovarian cases) and three heifers (with the cystic ovarian condition) from four different farms were selected for this study. The first and second farms consisted of Holstein (H) and Red Dane (RD) breeds respectively which were the progeny of imported Danish stock. The third and fourth farms consisted of dairy cows of first and second generation Holstein (HX), Jersey (JX) and Red Dane (RDX) cross-breeds evolved through the upgrading of Indian Zebu cattle. Every week, per rectal examination of genitalia of these animals was done for 3 to 4 months. Initially, cows possessing follicular structures of approximately 25 mm diameter with the absence of any palpable corpora lutea were considered as follicular cysts, Disappearance of softness and the presence of less resilient and thick walled structures were considered as cases of luteal cysts. The reproductive data of each animal, excluding four normal control cows is given in table 1.

Treatments given earlier to our study: Six months earlier to our study cow 79H had received 20 and 70 mg doses of stilboestrol i.m. for a presumable termina-

TABLE 1. The reproductive data of animals with cystic ovaries

Animal No.	Age in years	Lactation	Last Date of Calving	Date of oestrus prior to blood sampling	STATUS OF OVARIES		Outcome subsequent to experiment	Conception
					Before blood sampling —10 to —15 days	On the starting day of blood sampling Day 0		
					A	B	Regular oestrus	
27H	10	5	26-8-81	22-10-82	RO 3 cf—30 mm each	As in A	Yes	
31H	10	1	25-5-78	27-11-82	LO 4 cf—30 mm each	As in A		Yes RO
48H	9	7	15-11-82	8-12-82	LO 2 cf—30 mm each	As in A		Yes
79H	10	3	7-8-81	5-6-82	RO 4 cf } 30 mm each	As in A	Yes	
had double cervix					LO 3 cf }			
86H	10	3	25-3-79	18-8-82	RO 1 luteal cyst 50 mm	As in A		Yes LO
179H	6	4	11-10-82	2-11-82	RO 4 cf—30 mm each	As in A		Yes
309H	4	0		23-9-82	RO 3 cf—30 mm each	RO 3 luteal cysts 40 mm each	Yes	
Heifer								
294H	4	1	19-8-81	4-12-82	LO 3 cf—30 mm each	As in A		Yes
33RD	11	4	8-5-79	21-11-82	LO 3 cf—40 mm each	As in A		Yes RO
333RD	9	2	25-2-79	15-11-82	LO 3 cf—30 mm each	As in A		Yes RO
					RO 4 cf			Yes RO
254RD	6	0		7-11-82	LO 3 cf—30 mm each	As in A		Yes LO
Heifer								
415RD	7	0		10-11-82	RO 4 cf—30 mm each	As in A	Yes	
Heifer								
6HX	5	2	28-10-82	3-1-83	RO 4 cf—40 mm each	As in A	Yes	
4HX	5	2	18-11-82	8-12-82	RO 4 cf—30 mm each	As in A		Yes RO
8JX	5	3	21-7-82	20-12-82	RO 3 cf—30 mm each	As in A		Yes RO
P113HX	4	1	10-3-77	29-6-78	RO 4 cf—30 mm each	As in A		Yes
89H	11	2	10-4-77	7-10-82	RO 4 cf—40 mm each	As in A		Yes RO

RO — right ovary
LO — left ovary
cf — cystic follicle/s.

H — Holstein
J — Jersey
RD — Red Dane

HX — Holstein cross
JX — Jersey cross

tion of a mummy. The cow 86 H had received 3 intravenous injections of 5% lugol's iodine 20-25 ml, eight months earlier to our study. A month prior to this study, cow 8JX had received ethinyl oestradiol 0.12 mg plus megestrol acetate 20 mg i.m. on Day 2 of the previous oestrous cycle. The mother of cow 4HX

had gone through the cystic ovarian condition in each lactation and was concurrently cystic.

Blood sampling:

At weekly intervals, heparinised blood samples (5 ml; 10 i.u. heparin/ml; Heparin, Biological Evans, Bombay) were

collected through the jugular venipuncture. The blood sample was centrifuged at 500 g for 10 min and the separated plasma was stored at -15°C pending progesterone estimation.

Progesterone estimation by a radioimmunoassay procedure.

The progesterone concentration in the blood plasma was estimated by a radioimmunoassay procedure using a specific antibody (Sheela Rani & Moudgal, 1977). Progesterone was extracted from 100 μl plasma with 1 ml hexane. The extraction efficiency of tritiated progesterone was $74.5 \pm 4.6\%$ (n 10). Assaying a plasma sample of low progesterone concentration, the respective inter-and intra-assay coefficients of variation were 17.1 and 10.4% (n 10). Assaying a plasma sample of high progesterone concentration, the respective inter-and intra-assay coefficients of variation were 18.2 and 23.1% (n 10). The water blank for progesterone was 16.3 ± 3.9 pg/ml (n 5).

Results

The progesterone profile of individual cows is shown in Text-fig. 1. Among 17 animals with the cystic ovarian condition (Table 1), one cow, cow 31H showed a plasma progesterone concentration of less than 1 ng/ml throughout the period of study. In cow 79H, excepting for two times at which a plasma progesterone concentration of 1.3 ng/ml was noted, at other times, this cow resembled cow 31H in its progesterone profile.

The period of occurrence of low progesterone concentration in cows with the follicular cysts was variable (8 to 57 days range; 28.3 ± 5.3 s.e.m days) from cow to cow; it occurred between Day 0 to 40 in cow 89H, Day 0 to 14 in cow P113, Day 8 to 65 in cow 179H, Day 23 to 48

in cow 79H, Day 15 to 48 in cow 294H, Day 15 to 23 in cow 48H, Day 23 to 55 in cow 86H, Day 25 to 40 in cow 33RD, Day 17 to 40 in heifer 254RD, Day 8 to 27 in cow 8JX and Day 17 to 25 in cow 333RD.

The increased plasma progesterone concentration ($>2\text{ng/ml}$) noted in different cows at various time periods did not always coincide with the palpation of follicular cysts. Such increased progesterone concentrations were noted on Day 8 in cow 294H and cow 48H, Days 8-15 in cow 27H, Day 15 in cow 86H, Days 0-8 and 17 in cow 33RD, Days 0-8 in cow 333RD, Day 34 in cow 4HX and Day 0 in cow 8JX. Nevertheless, high plasma progesterone concentration was noted in animals with luteal cysts; it occurred on Days 0-10 in heifer 254RD, Days 8 and 20 in cow 6HX, Days 0 and 17 in heifer 415RD and Days 0 to 15 in heifer 309H.

The observed oestrus did always coincide with a low progesterone concentration. However, not in all cows blood samples were collected at the time of oestrus. In 3 cows (cow 31H, cow 79H and cow 4HX), oestrus occurred at intervals of 6 days. In two cows (cow 27H and cow 48H), the progesterone profile was similar to that of normal cows.

Subsequent to spontaneous regression of cysts, regular oestrous cycles but without conception occurred in heifers 309H and 415 RD, and cows 27H, 6HX and 79H, and with conception in 4 cows (cow 48H, cow 179H, cow 294H and cow P113 HX). There was spontaneous regression of cysts in 31.7×3.1 days; the regression was seen in Day 23 in cow 27H, cow 333RD (left ovarian follicular cyst), cow 6HX, heifer 309H and heifer 415RD, on Day 32 in cow 31H, cow

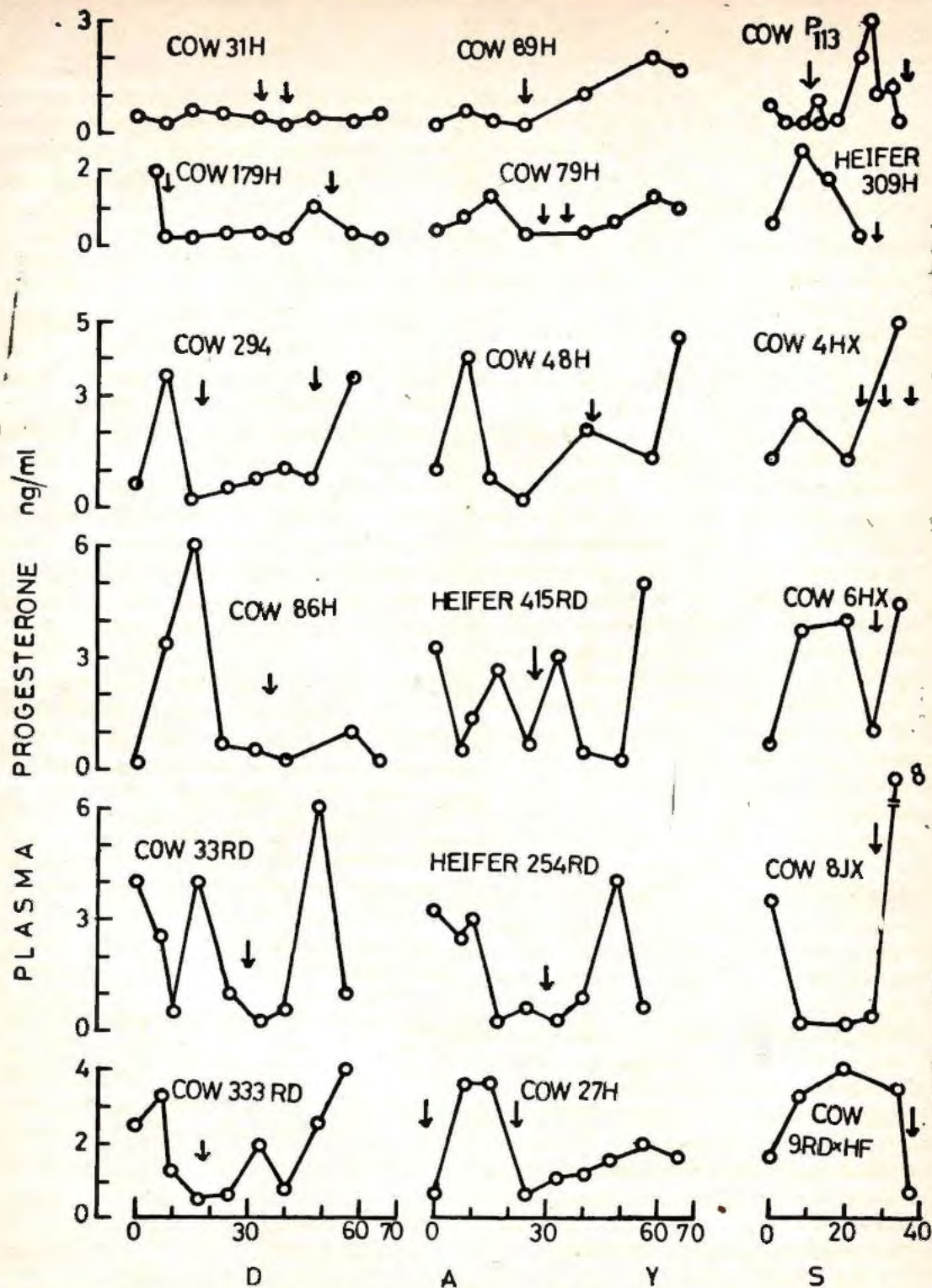


Fig. 1. The plasma progesterone concentration of dairy cows and heifers with ovarian cysts. The cow 9RD×HF is a control cow. The arrows denote oestrus.

86H, cow 89H, cow 333RD (right ovarian follicular cyst) and in cow 33RD, Day 47 in cow 48H and on Day 58 in cow 79H. The ovarian cyst persisted for more than 40 days in cow 4HX and cow 8JX. In 8 cows (cow 31H, cow 86H, cow 89H, cow 33RD, cow 333RD, heifer 254RD, cow 4HX and cow 8JX) the cysts recurred. The regression-recurrence interval was 19.1 ± 3.5 days; it was 8 days in cow 33RD, 10 days in cow 86H, 15 days in cow 31H, cow 294H and cow 333RD, 24 days in cow 79H and 33 days in cow 89H and heifer 254RD.

The transformation of the follicular cyst/s to luteal cyst/s occurred by Day 0 (Day 0 as per the graph and blood sampling day, that is 15 days after our first clinical examination) in heifer 309H, Day 8 in cow 6HX and Day 34 in cow 8JX. On Day 23, cow 8JX had cysts in both ovaries. In cow 333RD, on Day 37, only right ovary had follicular cysts. In three cows, the cysts reoccurred in the contralateral ovary (left to right ovary in cows 31H and 33RD and right to left ovary in cow 86H. The cysts reoccurred in the same ovary in cows 89H, 8JX and 4HX, and heifer 254RD.

Discussion

Similar to the report of Glencross and Munro (1974), in the present study, the plasma progesterone concentration was low in dairy cows with the follicular cysts. In eleven cows with follicular cysts, low progesterone concentration occurred for short intervals. At other times, eight of these eleven animals showed periodical or regular high plasma progesterone concentration and this is corroborative with the findings of Kesler *et al.*, (1980). Aberrant luteal tissues, either in the form of luteinised cystic follicles or small accessory corpora lutea

peculiar to cows with cystic ovaries (Tanabe & Brofee, 1982) or the possible diagnostic inaccuracies in per rectal examinations (Dawson, 1975) are presumably accountable for the wide variation in plasma progesterone concentrations noted in the present study. Nevertheless, high plasma progesterone concentration occurred in four cows with the luteal cysts.

Rather than anoestrus, oestrus, either at long or short intervals occurred in cows with the cystic ovaries.

Although, several workers (Wiltbank, Tyler & Casida, 1953; Morrow, Roberts, McEntee & Gray, 1966; Whitmore, Tyler & Casida, 1974) have reported spontaneous recoveries of cysts in some affected cows, the life span of cysts are unknown. In cows with corticotrophin-induced cystic follicles, Liptrap and McNally (1976) reported 13 to 17 days life span for cysts. The period of occurrence of low progesterone concentration (23 to 57 days) coupled with the absence of cysts at the palpated sites apparently reflect the life span of a follicular cyst.

The fate of the cysts could not be predicted from the preceding progesterone profile. Susequent to spontaneous regression of cysts, conception occurred in four cows and regular oestrous cycles with repeat breeder syndrome occurred in five cows. This occurrence of repeat breeder syndrome could have been circumstantial and appears to be independent of the pre-existing cystic condition.

In the present study, longer the post partum interval, presumably greater was the recurrence of cysts. However, it was not known when the cystic ovaries started in these cows and by the time the study was initiated, apparently many

recurrences could have occurred in these cows. Perhaps, greater the number of recurrences, permanent may be the infertility.

Either the recurrence of cyst/s in the same, contralateral or either ovaries, or the transformation of a follicular cyst to a luteal cyst suggests the dynamic nature of the cystic ovarian condition.

Prior to the onset of cystic ovaries, two cows had received oestrogens. Administering oestrogen on Day 16 of the oestrous cycle, Nadaraja and Hansel (1976) induced cystic ovaries in heifers. From the circumstantial findings in this study, it is not possible to conclude the apparent relationship between the previous treatment with oestrogens to the genesis of cystic ovaries. As per the reports of

Whitmore *et al* (1974) and Kirk, Huffman & Lane (1982), the concurrent occurrence of cystic ovaries in a dam and the daughter, although suggests the hereditary nature, further studies are needed to relate the occurrence of cystic ovaries to the heredity.

Acknowledgement

We are grateful to Dr. A. Ramu and other veterinarians of various farms for permission and co-operation in conducting the experiments. We thank Professor N. R. Moudgal for permitting us to use his radioimmunoassay facilities, Professor R. V. Patil and Dr. S. J. Seshadri, Director, for their co-operation and encouragement.

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Clinical Evaluation Of Clomiphene Citrate And A Combination Of Megesterol Acetate And Ethinyl Oestradiol For Treatment Of Anoestrus In Cattle

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ABSTRACT

Efficacy of clomiphene citrate ('Fertivet FVT 300') and a combination of megestrol acetate and ethinyl oestradiol ('Secrodyl') in the induction of oestrus in anoestrous cows and heifers was investigated. Nineteen cows and 33 heifers were administered 'Fertivet FVT 300' orally keeping 9 cows and 8 heifers as control. Twenty cows and 14 heifers were given 1 ml 'secrodyl' intra muscular for five consecutive days, while 8 cows and 6 heifers were kept as control.

'Fertivet' was capable of inducing ovulatory oestrus in 68.42 percent of cows and 63.64 percent heifers in 'true anoestrus', while 33.33 percent cows and 37.50 percent heifers came into heat in the control group. The difference between experimental and control animals among cows and heifers which evinced oestrus was statistically significant ($P < 0.05$). The conception rate in the induced heat was 42.11 percent in cows and 30.30 percent in heifers when treated with 'Fertivet' while only 11.11 percent of cows conceived in the control. Among heifers in the control group none conceived. Statistical analysis showed significant variation in conception rate between control and experimental animals among cows and heifers ($P < 0.01$). The over all conception rate by sub-

sequent insemination was 57.89 percent in cows and 42.42 percent in heifers while the corresponding figures in the control animals were 22.22 percent and 25 percent. This variation was also significant ($P < 0.05$).

Treatment with 'Secrodyl' could induce ovulatory oestrus in 50 percent cows and 50 percent heifers, while the response in the control group was only 25 percent among cows. None of the heifers in the control group showed ovulatory oestrus. This variation in the conception rate between experimental and control animals was significant. Significant variation ($P < 0.01$) in the conception rate between experimental and control animals was also observed, the values being 15 percent in cows and 14.28 percent in heifers in the treated group and 12.50 percent of cows and none in heifers among control group. The over all conception rate by subsequent inseminations also, showed significant variation between the two groups among cows and heifers.

* * *

Fertivet FVT 300 is a product of AR-EX Laboratories, Bombay containing 180mgm of Clomiphene citrate and 120 mgm of trans-clomiphene citrate per tablet (Anon, 1976).

TABLE 1. Results of treatment with 'Fertivet FVT 300' and 'Secrolyl'

Particulars	'Fertivet FVT 300'				'Secrolyl'				Fertivet total No. of animals	Secrolyl Total No. of animals
	Cow		Heifers		Cows		Heifers			
	Expt.	Cont.	Expt.	Cont.	Expt.	Cont.	Expt.	Cont.		
Total number	19	9	33	8	20	8	14	6	52	34
Number in which ovulatory heat induced	13 (68.42)	3 (33.33)	21 (63.64)	3 (37.50)	10 (50.00)	2 (25.00)	7 (50.00)	nil	34 (60.28)	17* (50.00)
Interval from commence- ment of treatment to oestrus in days	4.57 ± 0.16	22 ± 4.36	4.48 ± 0.21	5 ± 0.58	4.89 ± 0.72	26.5 ± 5.49	4.77 ± 0.15	—		
No. pregnant at First A.I.	8 (42.11)	1 (11.11)	10 (30.30)	—	3 (15.00)	—	2 (14.28)	—	18 (34.61)	5* (14.71)
Overall conception rate with subsequent A.I.	11 (57.89)	2 (22.22)	14 (42.42)	2 (25.00)	6 (30.00)	1 (12.50)	4 (28.57)	—	25 (48.08)	10* (29.41)

* Significant at 5% level

Figures in parenthesis denote percentage

** 'Secrolyl' is a product of Allenburys Bombay containing 5 mgm of megestrol acetate and 0.03 mgm of ethinyl oestradiol per ml.

Materials and Methods

Five hundred and seventy five cross bred cows and 446 heifers of the University Livestock farm and those brought for various infertility camps in the state formed the material for the study. Among these, 180 cows which failed to exhibit heat even after 60 days post partum and 165 heifers which failed to exhibit oestrus even after 24 months of age were identified as 'anoestrous' and subjected to detailed clinico-gynaecological examination at 10 day interval and 56 cows and 61 heifers having smooth inactive ovaries and without any cyclical activity were declared as in 'true anoestrus' and randomly subjected to the following treatment.

Nineteen cows and 33 heifers were administered orally with one tablet of 'Fertivet FVT 300' daily for five days

while 9 cows and 8 heifers were kept as control. Administration consisted of 125 ml of one percent copper sulphate solution followed by one pulverized tablet of 'Fertivet FVT 300' dissolved in 300 ml of water. The control animals were given only 125 ml one percent copper sulphate solution only daily for 5 days. Twenty cows and 14 heifers were given 'Secrolyl'** intramuscularly for five consecutive days and 8 cows and 6 heifers were kept as control.

After commencement of treatment experimental and control animals in both the groups were kept under close watch for manifestation of heat symptoms. Those showing behavioural signs of heat were confirmed by the presence of graafian follicle in the ovary. All the animals in the induced heat were inseminated with good quality chilled semen and those which failed to settle with first insemination were reinseminated at subsequent heat. Pregnancy was confirmed at 60 days after insemination. In both the groups if heat symptoms were

observed during the course of treatment further administration of the drug was stopped. The interval from the treatment to the onset of heat, percentage of animals which evinced heat in both the groups, percentage of animals which showed ovulatory oestrus and pregnancy rate were assessed and data analysed (Snedecor and Cochran, 1967).

Results and Discussion

The study revealed that out of 575 cows and 446 heifers, 180 cows (31.30 percent) and 165 heifers (36.99 percent) were reported to be 'anoestrous' beyond 60 days post-partum and 24 months of age respectively, though detailed examination could reveal only 56 cows (9.74 percent) and 61 heifers (13.68 percent) were in 'true anoestrus'. From this it is evident that, though, the reported anoestrous condition in the above bovine population was high, wide variation exists in the actual incidence of 'true anoestrus'. This variation in the cows reported to be in anoestrus and the true functional status based on the gynaecological examination may be attributed to the high incidence of silent heat during post partum period in cows and pubertal period in heifers as reported by Roberts (1971); Kruif (1977) and Luktuke and Sharma (1978). The incidence of 'true anoestrus' in the present study and those reported by previous workers in cows and heifers (Luktuke and Sharma 1978; Patel and Khan, 1978; Ansari, 1978; Mathew and Namboodiripad, 1979 and Ghosh, 1980) Showed wide variation which clearly indicated that managemental conditions and level of exotic inheritance definitely influenced the incidence.

Results of treatment of anoestrous animals with 'Fertivet FVT 300' and

'Secrodyl' are furnished in table 1. Out of 19 cows treated with 'Fertivet FVT 300' ovulatory oestrus was observed only in 13 (68.42 percent) at an average interval of 4.57 ± 0.16 days. While only 3 out of 9 (33.33 percent) in the control animals evinced oestrus within a mean period of 22 ± 4.36 days. This variation was statistically significant ($P < 0.05$). Among heifers, out of 33, only 21 (63.64 percent) showed oestrus within a mean period of 4.48 ± 0.21 days while only 3 out of 8 (37.5 percent) exhibited oestrus within a mean period of 5 ± 0.58 days in the control animals. This variation was statistically significant ($P < 0.01$). This is essentially in keeping with the findings of Deshpande *et al.* (1976); Pendse *et al.* (1977); Kaikini *et al.* (1978a) and Manjunath, (1979). Pillai (1980) found that 100 percent of cows and 89.47 percent of heifers expressed oestrus when they were treated with 'Fertivet FVT 300' at the above dose level. The interval from the administration of the drug to the expression of oestrus varied from 3 to 5 days in cows and heifers. It was also observed that an average 4.57 ± 0.16 days and 4.48 ± 0.21 days were required to induce heat in cows and heifers respectively. Similar observations were made by Pendse *et al.* (1977); Kodagali *et al.* (1978); Dugwekar *et al.* (1980) and Pillai (1980). Contrary to the favourable results of 'Fertivet FVT 300' in the induction of oestrus, Chauhan and Singh (1979) reported a poor response on heat induction in cows and heifers with deep anoestrous condition.

The present study also revealed that among 19 treated cows, 8 (42.11 percent) conceived at the induced heat while only one (11.11 percent) conceived at the induced heat in the control group. This variation in the conception rate

was statistically significant ($P < 0.05$). Among heifers, the percentage of conception was only 30.30 percent while none conceived in the control group. This is consistent with the finding of Kodagali (1978), Manjunath (1979) and Pillai (1980) in cattle and Hukeri *et al.* (1979) in buffaloes.

The overall conception rate in cows and heifers after subsequent insemination was 57.89 percent and 42.42 percent respectively as against 22.22 percent and 25 percent in control group. This tends to indicate that 'Fertivet' would correct the anoestrous condition of cows and heifers and bring them into normal cyclic activity as reported by Deshpande *et al.* (1976), Kaikini *et al.* (1977) Pendse *et al.* (1977), Dugwekar *et al.* (1980) and Pillai (1980).

Among 20 cows treated with 'Secrodyl' 10 (50 percent) expressed ovulatory oestrus within a mean period of 4.89 ± 0.72 days after the commencement of treatment. In the control animals out of 8 cows, only 2 (25 percent) evinced oestrus within a mean period of 26.5 ± 5.49 days. This difference in the expression of oestrus between experimented and control animals was significant ($P < 0.01$). Among heifers treated with Secrodyl, out of 14 only 7 showed ovulatory oestrus within a mean period of 4.77 ± 0.15 days while none of the control animals showed oestrus during the period of study. This difference was also significant ($P < 0.01$). Favourable results of 'Secrodyl' in the induction of oestrus in cows and heifers was reported by Gupta, (1980); Prasad *et al.* (1983) in cows and Umashankar and Verma (1982) in buffaloes. However, Tripathi *et al.* (1979) considered that, though, it might be possible to induce oestrus by parenteral administration of small doses of

oestrogen and Progesterone, the occurrence of ovulation and restoration of normal pattern of oestrous cycle could not be guaranteed. The study also revealed that among 20 cows treated, three, (15 percent) conceived at the induced oestrus and the overall conception rate by subsequent insemination was 30 percent. In the control animals the overall conception rate was only 12.5 percent. The conception rate at the induced heat was 14.28 percent in heifers while subsequent insemination resulted in the overall conception rate of 28.57 percent. The variation in the conception rate between treated and control animals was significant in cows ($P < 0.05$) and in heifers ($P < 0.01$). Though, the conception rate was found to be significantly higher in the experimental than in control animals, it was evident that the fertility rate was comparatively low when treated with Secrodyl. The present findings are in agreement with that of Gupta (1980) and Prasad *et al.* (1983) in cattle and Umashankar and Verma (1982) in buffaloes.

Comparing the efficiency of these two drugs in the induction of oestrus in anoestrous cattle, it was evident that the percentage of animals showing ovulatory oestrus was significantly higher (60.28 percent) when treated with 'Fertivet FVT 300' than those treated with 'secrodyl' (50 percent). Similarly, the conception rate was also significantly higher (34.61 percent) at the heat induced by "Fertivet FVT 300" than that induced by Secrodyl (14.71 percent). The overall conception rate of animals when treated with 'Fertivet FVT 300' (48.08 percent) was also higher than those treated with Secrodyl (29.41 percent).

Thus it could be inferred that both

'Fertivet FVT 300' and 'Secrodyl' were effective in the treatment of anoestrus in cows and heifers. But it appeared that, 'Fertivet' is superior in inducing oestrus with satisfactory fertility.

Acknowledgement

The authors are thankful to the Dean, College of Veterinary and Animal Sciences for permission to publish this paper.

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Glucose Concentration In Uterine Secretions Of Buffaloes During Certain Reproductive Phases

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ABSTRACT

The glucose concentration in uterine secretions of buffaloes during pro-oestrus, oestrus, dioestrus and early pregnancy was studied. There was cyclical variation ($P \leq 0.01$) in its concentration during oestrous cycle. The concentration of glucose was significantly higher ($P \leq 0.01$) in dioestrus than in pro-oestrus and oestrus. In early pregnancy its concentration was significantly lower ($P \leq 0.01$) than dioestrus and significantly higher ($P \leq 0.01$) than oestrus. The glucose concentration was 112.27 ± 4.07 , 48.83 ± 5.64 , 246.27 ± 13.12 and 97.73 ± 8.82 mg/100 ml during pro-oestrus, oestrus, dioestrus and early pregnancy respectively. The influence of gonadal hormones on glucose concentration in uterine secretions is discussed.

* * *

The recognition of uterine secretions as an important medium for sperm capacitation and development of fertilized ova has led to investigate more about its chemical nature qualitatively and quantitatively. However, there are no reports in the available literature regarding the glucose content in uterine secretions of buffaloes. As glucose is a substrate of energy for all the cells, knowing its physiological concentration during different phases of reproduction may possibly help in correcting infertility problems associated with the uterine

environment. Hence, a study was made on the glucose concentration in buffalo uterine secretions.

Materials and Methods

A total of 45 nonpregnant and 15 early pregnant (25 to 30 days) uteri of healthy buffaloes were collected from the abattoir. The nonpregnant uteri were categorized into pro-oestrus, oestrus and dioestrus based on the gross morphology of the ovaries (Choudary *et al.*, 1968; Abul Fadle *et al.*, 1974). The early pregnant uteri were grouped based on the biometry of the conceptus as per the technique of Arthur (1968).

The uterine secretions from nonpregnant uteri were collected by adopting the method of Olds and VanDemark (1957) with slight modification. Instead of hand operated clothes wringer, a wooden roller pin was used in the collection technique. The uterine secretions from pregnant uteri were collected similarly after removing the conceptus by making an incision at the os internus. The samples were immediately analysed for glucose content by adopting the method of Nelson and Somogyi as described by Annino (1964). The data was analyzed as per the method of Steel and Torrie (1960).

Results

The glucose concentration was 112.27 ± 4.07 , 48.83 ± 5.64 , 246.27 ± 13.12

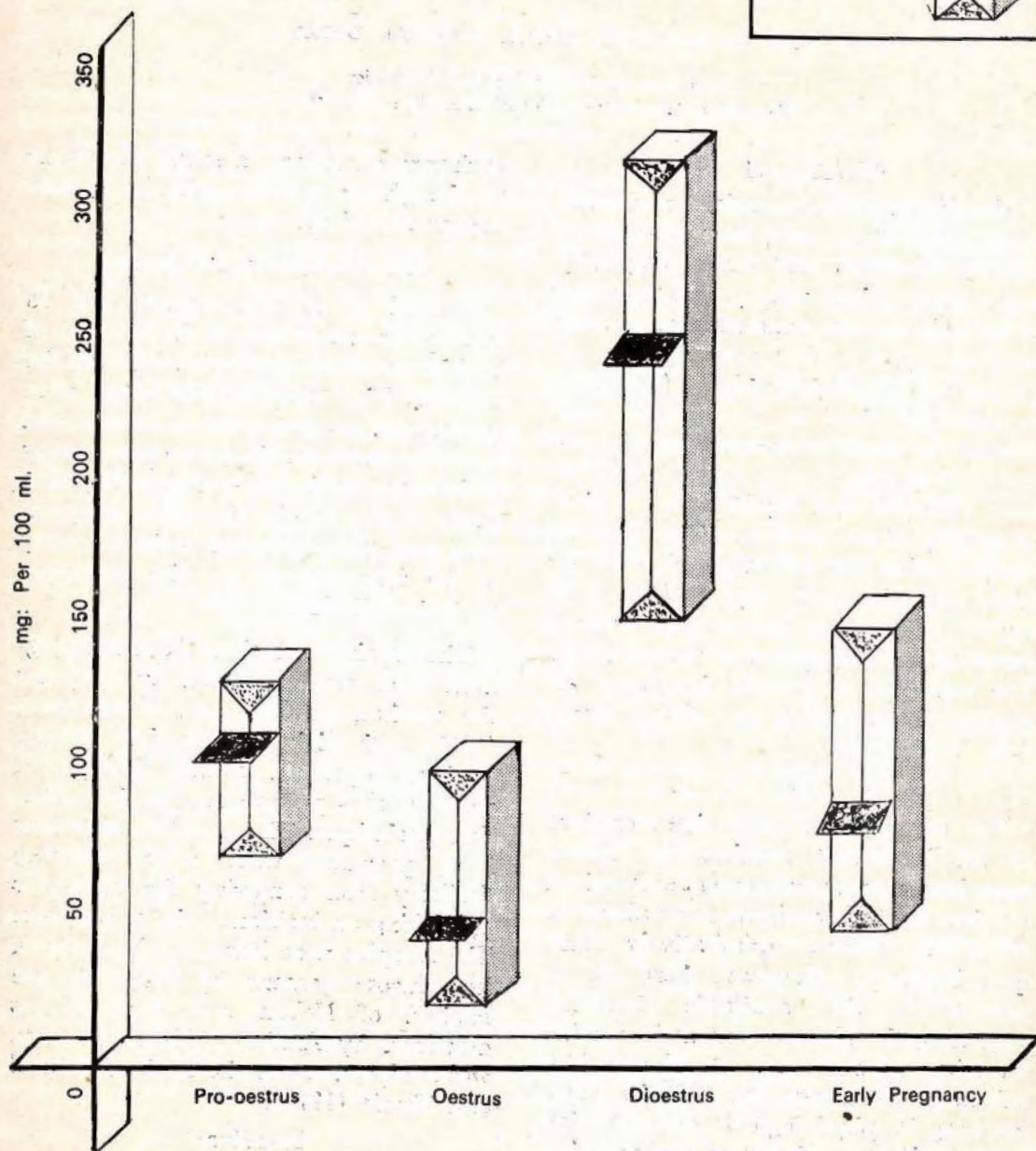
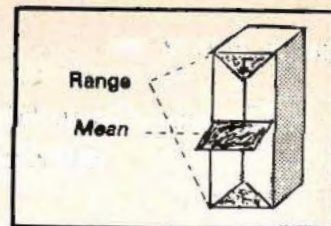


Fig. 2. GLUCOSE CONCENTRATION IN UTERINE SECRETION OF BUFFALOES DURING PRO-OESTRUS, OESTRUS, DIOESTRUS AND EARLY PREGNANCY

TABLE 1: Glucose concentration (mg/100 ml) in uterine secretions of buffaloes during certain phases of reproduction.

Reproductive phase	n	Mean	S.E.	Range
Pro-oestrus	15	112.27 ^a	4.07	70.50 to 131.00
Oestrus	15	48.83 ^b	5.64	18.00 to 100.00
Dioestrus	15	246.27 ^c	13.12	152.00 to 308.00
Early pregnancy	15	97.73 ^a	8.82	48.00 to 152.00

Means with different superscripts varied significantly ($P \leq 0.01$)

Means with common superscripts varied significantly ($P \leq 0.05$)

and 97.73 ± 8.82 mg/100 ml during pro-oestrus, oestrus, dioestrus and early pregnancy, respectively. There was significant ($P \leq 0.01$) variation between the phases of oestrous cycle studied. The concentration was highest during dioestrus and lowest during oestrus. In early pregnancy the concentration was significantly ($P \leq 0.01$) lower than dioestrus (Table-1, Fig. 1).

Discussion

The recorded values are similar to the finding of Schultz *et al.* (1971) in cows and the cyclical variation noticed are in agreement with Olds and VanDemark (1957).

Haynes and lamming (1967) have observed glucose as the major sugar in the uterine flushings of rat, rabbit, cow and ewe.

Glycogen synthesis in the endometrial cells appear to be under the influence of oestrogen (O'Malley 1971). The release

of glycogen and hydrolytic enzyme into uterine secretion is progesterone dependent (Henzl *et al.*, 1972). The cyclical variation observed in the present study may presumably be on account of the levels of ovarian steroid hormones.

During development, the nutritive requirement of the blastocyst especially before implantation is more than the stored nutrients in the ovum (Hugget and Hammond, 1967). The embryo survival was significantly correlated with the concentration of glucose in the embryotroph (Kovalenko, 1972). The uterine milk supplies the nutrients to the embryo prior to implantation (Liggins, 1975). These findings indicate that embryo utilizes glucose from the uterine secretions for its development.

The lower concentration of glucose in early pregnancy than in dioestrus observed in the present study may be due to the utilization of glucose by the developing conceptus.

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Serum Protein, Inorganic Phosphorus And Blood Glucose In Relation To Different Phases Of Reproduction In Crossbred Cattle

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ABSTRACT

Biochemical profile with regards to blood glucose, total serum protein, inorganic phosphorus level in normal cyclic, anoestrus, repeat breeding, normal calving and in retained placenta cases of crossbred cows was studied. The results revealed that blood glucose was significantly lower in anoestrus animals. Serum protein was significantly higher in repeat breeding animals. In retained placenta cases blood glucose, serum protein and inorganic phosphorus levels were found significantly lower than normal calvers.

* * *

Anoestrus and repeat breeding are two major gynaecological problems affecting economics of milk production in dairy animals. In post-partum animals the retention of foetal membranes has been reported as one of the important condition affecting the future fertility. The prevalence of these conditions in cross-bred animals have extensively been reviewed (Pandey, 1980; Prasad *et al.*, 1983 and Kharche *et al.*, 1982). Informations regarding biochemical attributes during these conditions in crossbred females seems to be scanty. In present paper an attempt has been made to study the level of blood glucose, serum protein and inorganic phosphorus

in relation to different phases of reproduction in crossbred cattle.

Materials and Methods

The study was conducted on 45 crossbred cattle of Livestock Production Research (Cattle & Buffaloes) of Indian Veterinary Research Institute, Izatnagar (U.P). All the animals were examined gynaecologically to ascertain the status of genital organs. On the basis of above examination the animals were categorised under the following groups.

Normal Cyclic

Ten animals exhibiting physiological oestrus evidenced by the presence of mature graafian follicle in the ovary formed this group.

Anoestrus

Seven cows which had not shown any sign of oestrus since last two months and had smooth non-functional ovary comprised this group.

Repeat breeding

Thirteen animals were included in this group which were bred for more than three times in successive oestrus with fertile bull semen but failed to conceive.

Normal calving

Seven cows which did not have any

Biochemical profile for Blood glucose, total serum protein and Inorganic phosphorus in Normal cyclic, Anoestrus, Repeat breeder, Normal calving and Retained placenta cows. (Mean \pm Standard Error)

Biochemical profile	Normal cyclic	Anoestrus	Repeat-breeder	Retained placenta	Normal calving
Total serum protein (g%)	8.43 \pm 1.22	8.62 \pm 1.52	**9.39 \pm 0.63	**8.53 \pm 0.72	9.81 \pm 0.51
Blood glucose (mg%)	84.54 \pm 13.37	*62.90 \pm 10.55	78.08 \pm 7.56	*66.75 \pm 8.15	76.57 \pm 4.23
Inorganic phosphorus (mg%)	8.19 \pm 1.93	8.17 \pm 0.71	8.03 \pm 0.82	**7.36 \pm 0.82	8.64 \pm 0.39

* Significant 5% level

** Significant 1% level

calving complications during or after parturition were kept under this group.

Retained placenta

Eight cows which could not expell the foetal membranes even after 12-18 hours post calving were included in this group.

Blood samples from each animal were collected from the jugular vein and processed for the estimation of blood glucose, serum inorganic phosphorus and serum protein as per the method of Follin-Wu (1920), Frankel *et al.*, (1970) and Oser (1965) respectively.

Results and Discussion

From the results it is apparent that the blood glucose level was significantly lower in anoestrus animals as compared to normal cyclic and repeat breeders. However, the difference in normal cyclic and repeat breeder was non-significant. Anoestrus condition may be due to low blood glucose because of some disturbances in the energy metabolism and utilization. Similar observations were recorded earlier (Sharma *et al.*, 1983).

Total serum protein value was significantly higher in repeat breeders than normal cyclic while in other two groups the difference was non-significant. Deficiency of protein may cause reproduc-

tive disturbance in animals (Arzumanjan and Dorojuk, 1964). The finding of present study with regards to serum protein in repeat breeders than normal cyclic is not in agreement with the earlier reports of non-significant difference in anoestrus, cyclic and repeat breeding cattle (Sharma *et al.*, 1983). Serum phosphorus level in repeat breeders and anoestrus was found slightly lower than that of normal cyclic cows but the differences were non significant. The findings are very much in agreement to Srivastava *et al.*, (1981). A significant decrease in inorganic phosphorus in infertile cows as compared to fertile cows has been reported (Ram Narayan, 1973).

In cows with retained foetal membranes blood glucose, serum protein and inorganic phosphorus were found significantly lower than the normal calvers which corroborates the finding of Boitor (1972). Glucose being major energy producing source may attributes to factors responsible for retained placenta besides the other factors. Lower level of inorganic phosphorus in cases of retained placenta than normal calvers has also been reported (Garbalik and Balon, 1978).

Acknowledgement

The authors are thankful to Dr. P. N. Bhat, Director, I.V.R.I., Izatnagar and

1/C LPR (C×B) for the facilities provided during the study. They are also thankful to Shri Rajendra Prakash, Scientist, S-1,

Division of Livestock Economics and Statistics for the analysis of the data.

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Concentration Of Iron And Zinc In The Cervico-Vaginal Mucus Of Fertile And Repeat Breeder Cows

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ABSTRACT

The concentration of Zinc and Iron was assayed in cervico-vaginal mucus of 18 fertile and 14 repeat breeder cows using Atomic Absorption Spectrophotometer. Neither of the two elements investigated differed significantly between the two groups. Furthermore, there was a wide individual variation in their concentrations and hence no definite relationship between their levels in the cervicovaginal mucus and fertility status of the cows could be established.

* * *

Cervical mucus has been recognised as a medium whose chemical composition has a profound influence on the survival of spermatozoa in the female genital tract and fertility. Several investigators have attempted to correlate the concentrations of various organic and inorganic salts, proteins, carbohydrates, lipids and enzymes in the cervical mucus with the fertility status of the cows in oestrus (Gupta, 1962; Linford, 1974; Reddy and Khan, 1976; Krishnaswamy and Uthappa, 1983). Although trace elements are important as co-factors, as activators of enzymes or as stabilizers of secondary molecular structure (Valee and Wacker, 1976) and concomitant infertility is believed to be associated with the enzymatic dysfunction associated with trace

element deficiencies (Hideroglou, 1979) the levels of various trace elements in the luminal fluids of the cow and their relationship with fertility is yet to be explored. Therefore, it is the objective of this paper to report the levels of iron and zinc in the cervico-vaginal mucus of fertile and infertile cows.

Materials and Methods

This study utilized 32 cross-bred cows categorized into the following two groups:

- a) *Fertile group cow*: Consisting of 18 cows inseminated for the first time during their first or second postpartum oestrous and conceiving to that insemination. Mucus samples were obtained before insemination.
- b) *Repeat breeder cows*: Consisting of 14 cows cycling regularly with apparently normal genitalia, but failing to conceive to the previous 3-5 inseminations with good quality semen. Cervico-vaginal mucus was aspirated from the anterior vaginal floor of each cow using a 10 ml pipette attached to a 20 ml glass syringe with a rubber adaptor.

For analysis of Iron and Zinc concentrations, one ml of cervico-vaginal mucus was digested in a di-acid digestion mixture consisting of perchloric acid and nitric

acid in the ratio of 1:4. The samples were digested on hot plate and the resultant residue was made up to a volume of 10 ml using double glass distilled water. Aliquots from each samples were then analysed for the concentrations of Iron and Zinc using Atomic Absorption spectrophotometer.

Results and Discussion

The data presented in TABLE-I show that there were no significant differences

TABLE 1. Mean Concentrations of Iron and Zinc in the Cervico-Vaginal Mucus ($\mu\text{G/ML}$) of Fertile and Repeat Breeder Cows.

Animals	Iron	Zinc
Fertile (N=18)	8.50	8.57
Repeat breeder (N=14)	1.06	0.97

in the concentrations of Iron and Zinc in the cervico-vaginal mucus of fertile and repeat breeder cows. In 5 out of the 18 cows in the fertile group and 3 out of 14 repeat breeder cows, the Iron concentration in cervico-vaginal mucus were below detectable levels in 3 fertile and 2 repeat breeder cows. The concentrations of Iron and Zinc showed wide variations between individual animals in both the groups. Therefore, the present study could not establish any relationship between the concentrations of Iron and Zinc in the cervico-vaginal mucus and the fertility status of the cow.

Although, many experimental and field studies in dairy cow have emphasized the importance of dietary trace elements in reproduction and have found a positive correlation between the concentrations of iron and zinc in blood plasma and the fertility status of the dairy cow (Lewis and Rolston, 1953; Korovina and Morozova, 1976; Manickam et al., 1977; Hideroglou, 1979), yet, there is no evidence to suggest that there is any correlation between the dietary or blood trace element composition and the concentration of trace elements in the cervical mucus. Cervico-vaginal mucus, by itself, is uniquely different from blood or seminal plasma in that its biochemical composition vary significantly not only between the different phases of the oestrus cycle, but also within the different stages of oestrus influenced by the changing concentrations of oestrogen and progesterone. Therefore, it is only natural that the concentrations of Iron and Zinc varied between individual animals in each group of cows under study, even though an attempt was made to collect the cervico-vaginal mucus at a constant period after the onset of oestrous. The preliminary results of this study, therefore, indicate that estimation of Iron and Zinc in the Cervico-vaginal mucus do not provide a clear picture of the fertility status of the cow. Alternate, it is possible that under the conditions of this experiment, the cause of repeat breeding was different.

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Biochemical Studies And Progesterone Levels During Placental Retention And Vaginal Prolapse In Buffaloes

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ABSTRACT

During retention of placenta and vaginal prolapse in buffaloes, the mean blood plasma values of total proteins; alkaline phosphatase; cholesterol; copper; zinc; iron and progesterone were observed as 6.67 ± 0.18 ; 8.20 ± 0.64 g/dl; 9.26 ± 0.25 ; 8.14 ± 0.36 kk units/dl; 235.60 ± 18.79 ; 203.06 ± 5.40 mg/dl; 146.94 ± 18.79 ; 73.38 ± 11.86 μ g/dl; 358.00 ± 16.45 ; 342.00 ± 26.40 μ g/dl; 304.15 ± 22.50 ; 192.59 ± 19.46 ; 0.92 ± 0.03 and 1.45 ± 0.23 ng/ml respectively.

The cause of high mean plasma values of total proteins, cholesterol and progesterones in retained foetal membrane (RFM) and vaginal prolapse (VP) are discussed.

* * *

Retained foetal membranes (R.F.M.) and Vaginal Prolapse (V.P.) constitute a major problem of reproduction and subsequently of economic importance in many herds of buffaloes. Few workers have studied the biochemical and normal changes associated with retention of placenta and vaginal prolapse in cows (Garkacik and Balon, 1978; Said *et al.*, 1964; Vujovic *et al.*, 1976; and Agthe and Kolm, 1975) but very little work has been reported in buffaloes.

The present study was designed to find out the changes which occur in the

blood of buffaloes following RFM and VP.

Materials and Methods

The study was conducted in clinical cases of retained placenta and vaginal prolapse brought to the clinics of the College of Veterinary Sciences, Pantnagar. Four animals were available for each condition.

The blood samples were collected from the jugular vein of the animals before resorting to any treatment. The blood was collected in heparinised clean glass serum test tubes and plasma was separated by centrifugation at 2000 rpm for 15-20 minutes and stored in clean glass vials at -20°C in a deep freeze till analysed.

Blood plasma samples were analysed for different blood constituents by the methods described by various workers as follows: Total proteins-Henry *et al.*, (1957); Alkaline phosphatase-Kind and King (1954); Total cholesterol-Zak (1957); copper-Gubler *et al.*, (1952); Zinc by the atomic absorption spectrophotometrics method of Davies *et al.*, (1968), Iron by the method of Henry *et al.*, (1957) and Progesterone by the competitive protein binding assay (Murphy, 1967).

The values of the above parameters in RFM & VP were compared with the

TABLE 1: Values of Various Blood Plasma Constituents in Obstetrical Conditions, Alongwith Means and Standard Errors

Sl. No.	PLASMA CONSTITUENTS	OBSTETRICAL CONDITIONS			
		Range	Mean \pm S.E.	Range	Mean \pm S.E.
		Retention of Fetal Membrans		Vaginal Prolapse	
1.	Total proteins (g/dl)	6.9—7.0	6.67 \pm 0.18	6.6—9.7	8.20 \pm 0.64
2.	Alkaline Phosphatase (K units/dl)	8.24—10.30	9.26 \pm 0.25	7.60—8.84	8.14 \pm 0.36
3.	Cholesterol (mg/dl)	205.48—285.97	235.60 \pm 18.79	189.08—211.58	203.06 \pm 5.40
4.	Copper (μ g/dl)	109.68—179.29	146.94 \pm 18.79	54.78—104.31	73.38 \pm 11.86
5.	Zinc (μ g/dl)	320—400	358.00 \pm 16.45	280—400	342.00 \pm 26.40
6.	Iron (μ g/dl)	242.71—345.64	304.15 \pm 22.50	146.23—240.20	192.59 \pm 19.46
7.	Progesterone (ng/ml)	0.80—1.00	0.92 \pm 0.03	0.78—2.00	1.45 \pm 0.27

reported values following normal parturition.

Results and Discussion

Data on biochemical and hormonal constituents estimated in R.F.M & V.P. are presented in Table 1.

Total Proteins:

The values of total proteins found in the present investigation in both RFM & VP were higher than the values reported in normal parturient buffaloes (5.86 gm %) by Seshagiri *et al.*, (1979). These high levels of total proteins may be attributed to the increase in the level of oestrogen and progesterone hormones in such conditions. An increased level of oestrogen in R.F.M. cases had been reported by Agthe and Kolm (1975) and of progesterone by Matton *et al.*, (1979) which agreed with the values in the present investigation for progesterone in RFM & VP cases. The increase in the levels of oestrogen & progesterone might be responsible for the increase in the total protein values in the present investigation as reported by Perk and Lobel (1960) for oestrogen and O'Malley and Mears (1974) for Progesterone.

Alkaline Phosphatase:

The levels of alkaline phosphatase in the R.F.M. & V.P. were observed to be lower which was in accordance with the findings of Kendall and Harshbarger (1962) and Sato (1978) who reported lower alkaline phosphatase values in cows few hours and few weeks respectively after parturition. In the present investigation the values for this enzyme were much lower than the reported values of Kendall & Harshbarger (1962). This could be due to the increased secretion of ovarian hormones in these conditions. The ovarian hormones had been reported to have depressing effect on enzyme levels. (Barker and Ludwick, 1967 and Baldwin *et al.*, 1969).

The total cholesterol levels found in RFM and VP were quite high and might be due to the presence of higher levels of circulating hormones at the time of parturition. Negative correlation of copper with cholesterol was reported by Harman (1970) in pigs. In the present investigation also low copper levels could be correlated with the high cholesterol level.

Copper levels were lower in the present investigation in both RFM and VP than

the values reported by Desai *et al.*, (1978) in buffaloes calving normally. Similarly low levels of copper were observed by Vujovic *et al.*, (1976) in RFM cases.

Zinc values found in the present investigation were within the range as reported by Khan *et al.*, (1979) in normal parturition which showed that Zinc has lesser role to play in obstetrical conditions. However, Dufty *et al.*, (1977) reported low zinc levels at parturition only in dystocia cases in cattle.

The Iron values were observed as 304.15 and 192.59 $\mu\text{g/dl}$ in RFM and VP respectively. Bostedt *et al.*, (1974) reported that there was a fall in iron level at and after parturition. But the values in the present investigation in RFM were quite comparable with the other reported values, however in V.P. there was a steep fall of iron level. The low level of iron in V.P. cases may be due to loss of blood as haemorrhage is quite common in long standing vaginal prolapse.

The level of progesterone in RFM and

VP cases was quite higher than the basal level reported by Agarwal *et al.*, (1980) in normal post parturient buffaloes. This high level of progesterone in RFM was in agreement with the findings of Agthe and Kolm (1975), Chow *et al.*, (1977) and Matton *et al.*, (1979). This high level of progesterone in RFM conditions could be preventing the normal separation of placenta from the maternal caruncles by suppressing the uterine contractions leading to retained after birth. In vaginal prolapse, higher values of progesterone needs further investigation. It is necessary to know the oestrogen values also in these RFM and VP cases to explain the role of higher progesterone values in causing these conditions.

Acknowledgement

The authors wish to thank the Dean, College of Veterinary Sciences, Director, Experiment Station, G. B. Pant University of Agriculture & Technology, Pantnagar and Director I.V.R.I., Izatnagar for providing the requisite facilities.

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Changes In Blood Profile From Antepartum, Parturition To Post-Partum Period In Anoestrous And Normally Reproducing Rathí Cows

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ABSTRACT

A high incidence of post-partum anoestrus in Rathí cows provoked to study the concentration of blood glucose, cholesterol, total serum protein, inorganic phosphate, calcium and magnesium were estimated at pregnancy, parturition and 1 month and 3 months post-partum in 9 anoestrous and 6 normally reproducing Rathí cows. Blood glucose level of anoestrous cows (37.00 mg/100 ml) was significantly lower than that of normals (46.33 mg/100 ml). In both the groups there was a rise in blood glucose level towards the time of parturition (55 mg/100 ml) and then a decline (40 mg/100 ml) at the post-partum period. At 3 months post-partum it again rose in animals that resumed normal cycling (46.33 mg/100 ml) whereas in anoestrous animals it further declined (37 mg/100 ml). Cholesterol level of anoestrous animals (181.61 mg/100 ml) was also significantly lower than that of normally reproducing cows (273.33 mg/100 ml). However, there was no significant difference in the levels of total serum proteins, inorganic phosphorus, calcium and magnesium between the two groups.

* * *

Rathí cows are well known native cattle of northern Rajasthan for milk production. Looking to the long service period and high incidence of anoestrous

condition which is an important attribute of non-infectious infertility in the large population of this breed inhabiting a vast area, the present study was undertaken. It was aimed to find the difference in the levels of blood glucose, cholesterol, total serum proteins, calcium, phosphorus and magnesium between the anoestrous and normally reproducing cows in order to elucidate the possible factors associated with the post-partum anoestrous in these animals.

Materials and Methods

Fifteen healthy cows of nearly same age and parity, in advanced stage of pregnancy were selected for the study from the herd of Rathí cows maintained at the College of Veterinary and Animal Science, Bikaner. Blood samples were collected at 8th month of gestation, at parturition and at 1 month and 3 month after parturition. Animals which exhibited oestrous within 60 to 90 days after parturition were treated as normally reproducing whereas those which did not come into oestrous even after 3 months and onwards after calving were considered to be anoestrous. Of the 15 cows, 9 were anoestrous and 6 were normally reproducing.

Estimation of blood glucose by the method of Nelson and Somogyi, cholesterol by Schonheimer and Sperry's method, total serum protein by the

method of Greenberg, serum inorganic phosphate by Fiske and Subba Row method, serum calcium by Clark—Collip method and serum magnesium by the method of Dennis were carried out as described by Oser (1976).

Results and Discussion

The average values of blood glucose, cholesterol, total serum proteins, inorganic phosphorus, calcium and magnesium of 9 anoestrous and 6 normally reproducing cows are presented in table 1.

Blood glucose:

Perusal of the results reveal an increase in the blood glucose concentration from advanced gestation to parturition in both the anoestrous and the control groups (Table 1). After calving with the onset of lactation the concentration of blood glucose declined at 1 month post-partum. After this point the level of blood glucose increased in the cows that resumed normal cycling (46.33 mg/100 ml) while it further declined in anoestrous cows (37 mg/100 ml) at 3 months post-

partum. Hypoglycaemia depresses hypothalamic function causing loss of ovarian activity due to the failure of release of gonadotrophins from the hypophysis (McClymont and Setchell, 1956; Wiltbank *et al.*, 1962; Howland *et al.*, 1966). Low level of glucose has been reported in infertile cows than in fertile cows by McClure (1965), Payne (1971), Downie (1976) and Parker and Blowly (1976). McClure (1968) reported that the oestrous cycle length was increased from normal to 34 days when cows were given insulin daily from day 17 through day 20 of an unmated oestrous cycle. Cessation of oestrous cycle was reported in normally cycling heifers by experimentally inducing hypoglycaemic state by injecting metabolic inhibitor 2-deoxy-D-glucose (McClure *et al.*, 1978). Present findings are in agreement with those of Patil and Deshpande (1979). Hypoglycaemia is the primary biochemical lesion responsible for infertility induced by energy deficiencies in lactating cattle (Bond *et al.*, 1958; McClure *et al.*, 1978).

TABLE 1: Average blood and serum profiles of anoestrous and normally reproducing cows at pre-partum, parturition and post-partum Period.

Blood & serum profile	Anoestrous cows				Normally reproducing cows			
	8th month gestation	Parturition	Post-partum 1 month	Post-partum 3 months	8th month gestation	Parturition	Post-partum 1 month	Post-partum 3 months
Blood glucose (mg/100 ml)	31.88	55.27	40.88	37.00	37.25	55.50	41.58	46.33
Blood cholesterol (mg/100 ml)	122.22	92.00	134.22	181.61	133.08	105.00	159.50	273.33
Total serum proteins (gm/100 ml)	8.24	6.10	7.68	7.60	8.42	6.36	7.95	7.93
Serum inorganic phosphorus (mg/100 ml)	5.66	5.12	4.72	5.12	5.83	5.03	4.86	5.13
Serum calcium (mg/100 ml)	12.41	11.28	10.42	12.26	12.73	11.30	10.18	12.55
Serum magnesium (mg/100 ml)	2.58	2.36	2.11	2.39	2.58	2.36	2.58	2.49

Blood cholesterol:

The concentration of cholesterol was significantly low in anoestrous cows than that of normally reproducing cows at all the 4 stages (Table 1). Murtza *et al.* (1978) also reported similar observations. At parturition the cholesterol level was lowest. After 1 month post-partum the level increased and at 3 months post-partum it further increased but this increase was much higher in animals that resumed early post-partum oestrous as compared to that of post-partum anoestrous animals. Lenon and Mixner (1957) and Jadhav *et al.* (1977) also reported similar findings.

Pregnenolone is the immediate precursor for all the steroids which is derived from cholesterol (Hafez *et al.*, 1980). Cholesterol in turn is synthesized from Acetyl-coA. Acetyl-coA is synthesized by oxidative decarboxylation of pyruvate and a few other reactions. Pyruvate in turn is the product of glycolysis. Therefore concentration of blood cholesterol and in turn steroid synthesis are possibly related to energy status of the animal. Subnormal energy status induces hypocholesterolemia which in turn may lead to improper output of steroids. Velhankar (1973) reported a positive correlation between blood cholesterol concentration and energy status of the animal. Higher cholesterol concentration and over all better reproductive performance was found in animals fed on 120 per cent NRC dietary energy level.

Total serum proteins:

The concentration of total serum proteins at advance gestation, parturition and puerperium in control and anoestrous cows revealed highest levels at 8 month gestation which declined at the time of parturition and then recovered after

parturition (Table 1). Anoestrous cows had a lower level than that of the normally reproducing ones but the difference was not significant. These findings are in agreement with those of Larson and Kendell (1957) and Humana and Ushi (1973).

Serum inorganic phosphorus:

The averages of serum inorganic phosphorus concentration of anoestrous and control cows are presented in table 1. Statistically there was no significant difference between the two groups. Phosphorus is necessary for energy metabolism of the body (Foley, 1952). Mild deficiency of phosphorus in the diet does not significantly reduce the serum inorganic phosphorus as it may be replaced from skeletal reserves, but extreme malnutrition may result into exhaustion of skeletal reserves and hypophosphatemia. It appears that when the levels go down to 3 mg/100 ml, external symptoms of phosphorus deficiency may result. Present findings are in accordance with those of Mahadevan (1961) and Samad *et al.* (1980).

Serum calcium:

Concentration of serum calcium at advanced gestation, parturition, 1 and 3 months post-partum in 9 post-partum anoestrous and 6 control cows indicate that the levels decline after parturition due to onset of lactation. No significant differences were found between the two groups. These observations are in agreement with those of Mahadevan (1961) and Samad *et al.* (1980).

Serum magnesium:

No significant differences were found in the serum magnesium concentration of anoestrous and control cows. There was a slight decline in the levels after parturition in both the groups (Table 1).

Acknowledgement

The authors are highly thankful to Dr. Mohan Singh, Dean, College of

Veterinary and Animal Science, Bikaner for providing the facilities.

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Investigations Into An Outbreak Of Abortion In Buffaloes Due To *Brucella Abortus*

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ABSTRACT

A severe epizootic of brucellosis causing abortions in buffaloes has been investigated at a private dairy farm, West Delhi. Of the 85 milch buffaloes of different breeds and age groups, 21 animals aborted at 6-9 months of pregnancy. The serological evidence of *B. abortus* antibodies was detected in 17 aborted buffaloes when examined by standard tube agglutination test. However, the organism could be isolated on bacteriological media from 3 placenta, 3 foetal lung, 2 abomasum and 1 vaginal discharge. Differential diagnosis and zoonotic significance have also been discussed.

* * *

Brucellosis is an important, infectious bacterial disease of animals and man; and recorded from many countries of the world including India (Mathur, 1968; Kulshreshtha *et al.*, 1978; Blood *et al.*, 1979). The diagnosis of brucellosis in animal is usually established on the basis of serological findings (Pietz and Cowart, 1980). The bacteriological cultural examination of the clinical materials, though imperative for confirmation, is not commonly attempted. The present investigation aims to establish the etiology of an abortion in buffaloes due to *B.*

abortus by employing microbiological and serological techniques.

Materials and Methods

An epidemic of abortion occurred in a private dairy farm situated in rural areas of West Delhi. There were about 85 buffaloes of Murrah and indigenous breed. In addition there were 37 buffalo calves of both sexes and 3 male buffalo bulls. The serum samples were collected obtained from 21 aborted buffaloes, 6 apparently healthy buffaloes, 3 buffalo bulls and 2 young heifers. The details regarding the age of the animal, number of lactation, duration of pregnancy, types of clinical symptoms etc. were recorded for each buffalo. The sera were separated after centrifuging it at 4,500 r.p.m. for 10 minutes, and kept at 4°C. *Brucella abortus* plain antigen for serological study was obtained from the Director, Indian Veterinary Research Institute, Izatnagar (U.P.). The standard tube agglutination test (SAT) was performed as recommended by Alton *et al.* (1975). All the sera samples were incubated in waterbath at 56°C for 30 minutes to eliminate anti-complementary activity of the serum. The serum was diluted with phenol saline in 50 × 12 mm round bottomed agglutination tubes. The dilution of the tube which showed 50% agglutination was taken as

TABLE 1: Prevalence of *Brucella abortus* antibodies in the sera of buffaloes

Source of sera	Total No. of sera tested	Demonstration of <i>Brucella</i> agglutinins titres in sera			
		1:40	1:80	1:160	1:320
Aborted buffalo	21	6	8	2	1
Healthy buffalo	6	0	0	0	0
Buffalo bull	3	0	0	0	0
Young heifer	2	0	0	0	0
Total	32	6	8	2	1

end point. The isolation of the bacteria from various clinical material such as placenta, foetal lung, abomasum, vaginal discharge were carried out by using standard techniques (Cruickshank *et al.*, 1975). The identification of *Br. abortus* was made according to the procedures laid down by various workers (Hausler and Koontz, 1974; Corbel *et al.*, 1979).

Results

Out of 85 buffaloes, 21 aborted in late gestation. The duration of pregnancy varied from 6 to 9 months. Most of the affected animals showed vulvar oedema and udder enlargement before abortion. The placental membranes were retained in 19 buffaloes. In 5 animals serve metritis developed after abortion, and infertility was seen in 4 cases. In most of the cases placentas were thickened, oedematous and necrotic and aborted foetus showed anasarca, enlargement of liver and pneumonia besides multiple patches in the skin.

In all 32 serum samples originated from same number of animals were investigated for the prevalence of *Brucella* agglutinins (Table 1). The standard tube agglutination test detected antibodies to *Brucella abortus* in 17 aborted buffaloes. The antibody titres in diseased animals ranged from 1:40 to 1:320 I.U. None of the clinically healthy buffaloes, young heifers and buffalo bulls showed any evidence of *Brucella* infection as

shown by the absence of significant antibodies titres in their blood.

Isolation of *Brucella abortus* was attempted on tryptose agar and serum dextrose agar from various clinical materials. Of the 43 specimens cultured, only 9 yielded pure growth of *Brucella* organism (Table 2). The remaining of the plates were

TABLE 2: Isolation of *Brucella abortus* from clinical specimens of 17 buffaloes

Type of clinical material	Number of specimens cultural	Number yielded growth of <i>Brucella</i>
1. Placenta	17	3
2. Foetal lung	11	3
3. Foetal abomasum	9	2
4. Vaginal discharge	6	1
Total	43*	9**

* These specimens were collected from 17 diseased buffaloes

** Positive isolations came from 7 animals

contaminated with fast growing micro-organisms. The bacterium appeared Grams positive, coccobacillary, non-motile, non-sporing, and catalase and oxidase test were positive. It produced H₂S and growth was inhibited in the presence of thionin. All the strains oxidized D-glucose, D-glactose, D-ribose and arabinose.

Discussion

The present investigation records an

epizootic of *Brucella* abortion in buffaloes during third trimester of pregnancy. The clinical, serological and microbiological observations confirmed that *Brucella abortus* was the sole causative agent involved in the etiology of this severe outbreak which resulted in abortion of 21 animals. Among various species of *Brucella*, *Br. abortus* is considered the chief etiological agent of bovine brucellosis (Blood *et al.*, 1979). However, the serological evidence of *Brucella* infection in buffaloes and other animals has been reported by many workers (Mathur, 1968; Kulshreshtha *et al.*, 1978; Halder *et al.*, 1979; Pal, 1982; Wisniewski and Ranus, 1984).

Our limited experience indicated that the isolation of *Brucella* organisms from the infected materials collected under field condition may not be easily attempted because of heavy contamination. Hence, the demonstration of *Brucella* agglutinins in the blood sera of diseased animals by serological techniques helps in the diagnosis of brucellosis. The same view has been expressed by others that serological methods are commonly used to diagnose brucella infection (Kulsh-

reshta *et al.*, 1978; Abu-Damir *et al.*, 1984). It has been emphasised that enzyme-linked immunosorbent assay (ELISA) should be employed for an early diagnosis of brucellosis to monitor the control programme.

It would be pertinent to mention that all cases of abortion in young buffaloes in first pregnancy with history of late gestation and retention of placenta should be prudently investigated for brucellosis. As the disease simulates clinically to leptospirosis, listeriosis, salmonellosis and mycotic abortion, the diagnosis must be supported by cultural and serological tests. Since brucellosis is one of the major zoonotic disease, care should be taken while dealing with the infected animals and their discharges to prevent human infection.

Acknowledgement

We wish to thank the staff of private dairy farm and Veterinary hospital, Delhi for their kind help and cooperation in the collection of clinical materials. The technical assistance of Mr. Ram Prakash is also gratefully acknowledged.

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Electrophoretic Characterization Of Follicular Fluid Proteins From The Goat (Capra hircus) Ovary

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ABSTRACT

Categories of proteins present in the follicular fluid of goat ovaries have been presented and their effects discussed.

* * *

The follicular fluid (FF) and its constituents are derived from blood and from the secretory and specific metabolic products of the granulosa cell. Owing to its proximity with the granulosa cells and the oocyte FF is considered to play a significant role in the maturation and function of these cells (Edward, 1974; Chang *et al.*, 1976; Peter and McNatty, 1980; and Gwatkin, 1980). Follicular fluid provides the means by which cells within an avascular region of the follicle can be exposed to an environment different from serum and the adjacent follicles. Changes in the physical and chemical properties of follicular fluid are necessary for follicular steroidogenesis, oocyte maturation, ovulation and transport of the oocyte to the oviduct, apart from its role in preparing the follicle for subsequent corpus luteum function (see Edward, 1974; McNatty, 1978; and Gwatkin, 1980). Therefore, the precise

understanding of chemical composition of follicular fluid is of great importance.

Any departure from normal in the chemical composition of FF may reflect altered metabolism within or around the follicle and may afford explanation to various dysfunctions like cystic ovaries, anovulation, fertilization failure and early embryonic death etc.

The present study was undertaken to characterise and compare the various categories of proteins present in the follicular fluid of small, medium and large sized follicles of goat ovary employing sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) including anodic and cathodic systems of electrophoresis. The results of these experiments will provide rationale for investigating the effects of follicular fluid and its components on the process of oocyte maturation, ovulation, capacitation and acrosome reaction.

Materials and Methods

Ovaries of the non-pregnant goats were obtained from the local slaughter house and transported immediately to the

laboratory in polythene bags packed in freezing—mixture. Ovarian follicles were classified into three categories according to their gross morphology and diameter viz, a) small follicle ($2 \leq \text{mm}$), b) medium follicle (2-5 mm) and c) large follicle (5 mm). The follicular fluid was aspirated using a syringe and 26 gauge needle.

FFs from 40-50 ovaries were pooled according to their size and centrifuged at $800 \times g$ 20 min at 4°C to remove the cellular debris. For comparison serum was also obtained from nonpregnant goat maintained at the university goat farm. The serum samples were also centrifuged at $800 \times g$, 20 min at 4°C . The resulting supernatants from FF and serum were frozen in polypropylene tubes. Samples were stored at -20°C until used.

Protein concentrations were determined by the assay described by Lowry *et al.* 1951; using bovine serum albumin (BSA) as a standard. The proteins present in the FFs and serum were fractionated by non-denaturing polyacrylamide disc gel electrophoresis on 7.5% gels for both anodic (pH 8.9 buffer system, Brewer and Ashworth, 1969) and cathodic (pH 4.3 buffer system, Reisfield, *et al.*, 1962) proteins. A 0.005% bromophenol blue and 0.1% of methyle green were used as marker dyes for the anodic and cathodic systems respectively. For

electrophoresis, 100-150 μl of each sample containing 75-100 μg protein was layered on the gel (Dave and Graves, 1976). Electrophoresis was carried out with 2 mA current per tube until the marker dye migrated to a distance of 5 cm in the gel. Gels were stained for proteins with Coomassie brilliant blue according to the method of Laemmli (1970) and were scanned densometrically at 560 nm in a Gilford 260 uv spectrophotometer equipped with chart recorder. The relative concentration of each protein fraction was determined using planimeter.

The molecular weights of the various proteins in FF fluid and serum were determined using denaturing system of SDS-PAGE according to the method of Laemmli (1970) using 10% polyacrylamide gels. Protein samples were also subjected to mercaptoethanol treatment before SDS-PAGE for breaking up of the disulfite bridges between different proteins. SDS-PAGE was carried out using 100 μl of sample containing 50-75 μg protein in 5 cm gels with 2 mA per tube. The gels were stained for protein by the method of Chrambsch *et al.*, (1967) and scanned densometrically at 530 nm. The areas under different peaks were calculated using planimeter. The molecular weights of the proteins were determined by using standard proteins such as catalase (2,32,000), phosphorylase b

TABLE 1. Total Protein Concentration in Individual Follicle

Follicle	Concentration (mg/ml)	% concentration of that in serum*
Small follicle	77.50	84.24
Medium follicle	63.63	69.16
Large follicle	65.34	71.02
Serum	92.00	—

$$* \% \text{ concentration of that in serum} = \frac{\text{Conc. in FF}}{\text{conc. in serum}} \times 100$$

TABLE 2. Concentration of Different Cathodic Proteins in Individual Follicle (expressed as a % of that in serum)

Proteins	Small follicle	Medium follicle	Large follicle
1-2	31.66	45.68	30.29
3	70.49	78.41	85.80
4	70.50	96.66	96.10
5	193.10	163.07	180.81
6*	40.60*	35.11*	24.29*

* Protein 6 is not found in serum and values are relative % ages in small, medium and large follicles.

(94,000), bovine serum albumin (68,000) ovalbumin (45,000) and trypsin (23,000).

Results

Protein concentration in FF from various-sized follicles and serum are summarized in Table I. The total protein concentration in all the categories of follicles remained relatively constant irrespective of their size. However, small

follicle showed slightly higher protein concentration as compared to medium and large follicles. The total protein concentration in the serum was higher than in the FF (see Table 1).

Nondenaturing electrophoretic fractionation of FFs from small, medium and large follicles employing both anodic and cathodic systems reveals that they have similar number of both anodic and

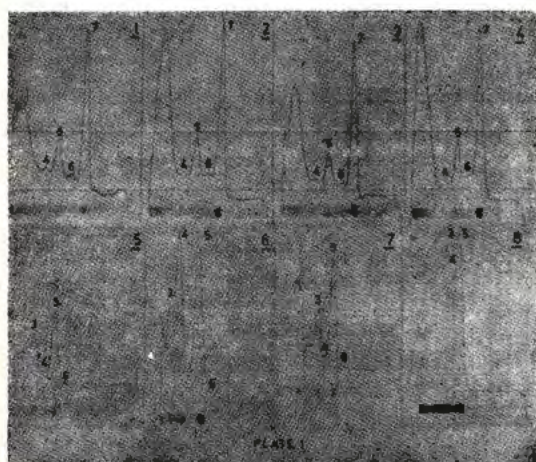


PLATE 1 Figures 1-4, anodic proteins on polyacrylamide gels with scans and figures 5-8, cathodic proteins on polyacrylamide with scans of follicular fluids from small follicle, medium follicle, large follicle and serum respectively.

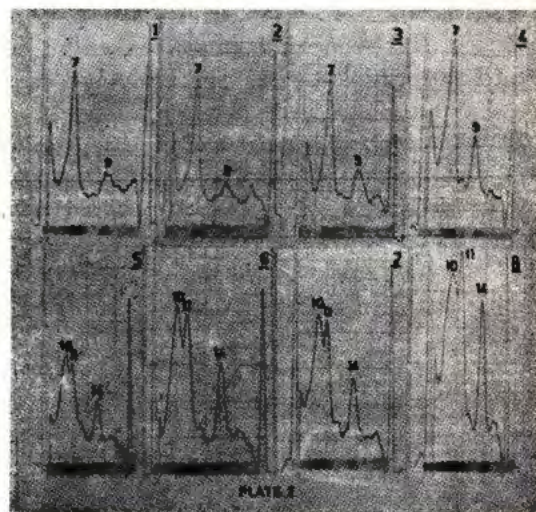


PLATE 2 Figures 1-4, SDS-PAGE minus mercapto ethanol with scans and figures, 5-8, SDS-PAGE plus mercapto ethanol with scans of follicular fluids from small follicle, medium follicle, large follicle and serum respectively.

TABLE 3. Concentration of different anodic proteins in individual follicle (expressed as a % of that in serum)

Protein	Small follicle	Medium follicle	Large follicle
1—4	118.49	111.91	112.78
5	133.80	136.77	136.87
6	63.34	74.07	45.02
7	97.46	95.12	100.97

cathodic proteins irrespective of their sizes. However, in cathodic system, a shoulder (designated as peak 6) on peak 5 was found only in FF and not in the serum (see Plate I, Figs. 5,6,7 and 8). The relative concentration of various cathodic and anodic proteins are represented in Tables 2 and 3 respectively. The major protein in FF was serum albumin varying between 55% and 58% of the total proteins in different sized follicles and is predominantly derived from the serum representing 95% to 193% of serum albumin. The serum albumin is designated as peak 5 and 7 in cathodic and anodic systems respectively (see

Plate 1). The concentrations of other anodic and cathodic proteins were comparable in different sized follicles. However, follicles selectively concentrate an anodic protein 5 (see table 3). A slow migrating anodic protein was present only in serum but not in FF.

Fractionation of follicular fluids from different sized follicles in denaturing system of SDS-PAGE in the presence or absence of mercaptoethanol yielded seventeen different proteins with different molecular weights (see Table 4), which include α -globulins. However, some of the proteins could not be identified as shown in the table 4. The relative concentra-

TABLE 4. Molecular weights of various proteins found in goat follicular fluid

Protein No.	Identification	Molecular weight
1	—globulins	2,32,000
2		2,20,000
3		1,90,000
4		1,83,000
5	Immunoglobulin	1,65,000
6	Plasminogen	1,45,000
7	?	1,32,000
8	β -globulin	84,000
9	?	74,000
10	Albumin	68,000
11		62,000
12	Prealbumins	54,000
13		50,000
14	—Globulins	45,000
15		40,000
16		37,000
17		25,000

TABLE 5. Concentration of different proteins in individual follicle (expressed as a % of that in serum) in sodium dedecylsulphate-polyacrylamide gel electrophoresis without mercaptoethanol

Proteins	Molecular weights	Small follicle	Medium follicle	Large follicle
1	2,32,000	83.88	62.38	83.09
2	2,20,000			
3	—2,25,000			
	1,80,000			
4	—1,90,000			
	1,30,000			
	—1,34,000			
5-6	74,000	87.43	113.32	169.92
	— 75,000			
7	64,000	93.14	108.90	86.02
	— 67,000			
8	51,000	547.41	370.37	428.39
	55,000			
9	25,000	40.65	43.68	45.82
9	25,000	40.65	43.68	45.82

tions of different molecular weight proteins in small, medium and large follicles are shown in Tables 5 and 6. The large molecular weight proteins in the range of γ -globulins ranging from 1,30,000 — 2,32,000 were fractionated in SDS-PAGE (see Plate 2, Figs. 1-8), however, these fractions were not read by Scanner as separate peaks, but were shown as shoulder to a major peak

succeeding them. Therefore, in Table 5, a pooled-value for these proteins has been given. These high molecular weight proteins tend to break up into their sub-units after treatment with mercaptoethanol before SDS-PAGE (see Plate 2, Figs. 5-8) and similarly, the concentrations of these proteins have been presented as pooled-values in different sized follicles (see Table 6). These large molecular

TABLE 6. Concentration of different proteins in individual follicle (expressed as a % of that in serum) in sodium dedecylsulphate polyacrylamide gel electrophoresis with mercaptoethanol

Proteins	Molecular weights	Small follicle	Medium follicle	Large follicle
1-9	1,00,000—	61.26	22.66	51.02
	—2,32,000			
10	82,300—	173.24	219.30	110.82
	— 87,000			
11	60,000	153.37	165.47	166.07
	— 67,000			
12	42,000—	62.62	87.56	56.42
	— 49,000			
13	35,000—	228.92	76.31	112.31
	— 49,000			
14	23,000—	76.72	90.79	65.44
	— 25,000			

weight proteins in the range of γ -globulin are present in higher concentrations in serum than in FFs.

Albumin in FF was present in almost similar concentration as in serum (see Table 5; protein 7). After treatment with mercaptoethanol the mobility of albumin slightly enhanced and the prealbumin fraction becomes predominant (see Plate 2, peak 7, Figs. 1-4; peak 11, Figs. 5-8) and concentration of albumin in follicular fluids seems to be higher than in serum (see table 6, protein 11).

The concentration of proteins of 82,300 to 87,000 molecular weight in the range of β -globulins were higher in FF than in the serum (see Table 6). FF from medium follicles accumulated more β -globulins than from small and large follicles.

Post albumin fractions of molecular weights 51,000 — 55,000 falling in the outer limits of α -globulins are selectively accumulated in the FF by all the follicles from the serum (see Table 5). These α -globulins after treatment with mercaptoethanol seemed to split up into slow moving α -globulins of molecular weight 42000 to 49000 and fast moving α -globulins of molecular weight 35000-49000 (See Table 6). The later α -globulins are accumulated in the FFs particularly in the small follicles. Another fast moving post-albumin fraction of molecular weights 25,000 was present in higher concentration in serum than in the FFs (See Table 5). This protein showed progressive increase in its concentration from small to large follicle. After treatment with mercaptoethanol, this protein of molecular weight 25,000 in all the FFs and also in serum gave very sharp peak, distinguishably sharper than before mercaptoethanol treatment (see Plate 2, Figs. 1.4; peak 9; Figs. 5-8, peak 14).

The relationship between the mean follicular fluid: serum concentration ratio and molecular weights of eight individual proteins from goat follicles showed a significant correlation ($r=0.74$, $p<0.01$) indicating that the mean follicular fluid: serum concentration ratio of eight proteins are inversely correlated to the molecular size.

Discussion

The analyses of follicular fluid proteins from goat ovary has provided much of the evidence revealing their partial equilibrium with serum. Most of the serum proteins are present in FF although the levels are different from those in serum. Our findings clearly show that the concentration of total proteins in FF is approximately 69.16-84.24% of that in serum which is comparable to the values, 64.1-86.4 for cow (Caravaglios and cilotti, 1957; Desjardins *et al* 1969; Pascu, *et al* 1971, Anderson, *et al*, 1976). However, in other studies, the concentration of protein in FF has been shown to be little lower or equal to serum e.g. (74.7-103% for pig: McGaughey, 1975; Chang *et al* 1976, 53.0-98.7% for human: Manarang-Pangan and Menge, 1971; Shalgi *et al* 1972). It is evident that FF from small follicle contained slightly higher protein concentration than that of medium and large follicle, which is nevertheless lower than that in serum. Pig FF has also revealed similar results (McGaughey, 1975). It is possible that partitioning of proteins between serum and FF is achieved relatively quickly followed by dilution of protein in the medium and large sized follicle.

Present study shows that FFs from small, medium and large follicles have similar number and comparable concentration of anodic and cathodic proteins. Most of these proteins are derived from

the serum. But in cathodic system a follicular specific pre-albumin protein was found in small, medium and large-sized follicles which was not detected in the serum. In human and bovine FF the only protein which has been shown to be present both in FF and plasma but not in serum is fibrinogen (Manarang-Pangan and Menge, 1971; Anderson *et al.*, 1976). The present study has also revealed the absence of a serum-specific anodic protein from the FF goat. This protein is possibly the β -lipoprotein, which is absent in the FF of many mammalian species (Johnson, 1973; Edward, 1974; McNatty, 1978). The most predominant protein of goat FF detected in both anodic and cathodic fluid and derived from the serum is the albumin as also reported in other species (See Edward, 1974; McNatty, 1978; Gwatkin, 1980). Paradoxically, albumins are present in lower concentrations in human and bovine follicular fluid (Zachariae and Jensen, 1958; Johnson, 1973).

The results obtained with denaturing SDS-PAGE in the presence and absence of mercaptoethanol using 5 cm gels, demonstrate that a minimum of 17 serum proteins are present in the FF. Similarly major serum proteins also occur in FF from different mammalian species as revealed with immunoelectrophoresis (Shalgi *et al.*, 1973; Edward, 1974; Anderson *et al.*, 1976; McNatty, 1978). α - and β -globulins-like proteins are present in higher concentration in FF of the goat than in its serum and reverse is true for γ -globulin-like proteins. The relatively lower amounts of α - and β -globulins in serum than in FF are also reported for cows (Caravaglios and Cilotti, 1957; Anderson *et al.*, 1976), human (Shalgi *et al.*, 1973) and pig (McGaughey, 1975). Desjardins *et al.*, (1966) demonstrated as

was also observed for the goat in the present study, that bovine serum contains higher relative amounts of γ -globulins than does bovine FF. The demonstration of slow and fast moving α -globulins in goat FF is similar to the observation of others (Takikewa, 1966; McGaughey, 1975). Albumin in goat follicular fluid as studied in SDS-PAGE is present almost in similar concentration as in serum. Similar observations have been made in bovine by Anderson *et al.* (1976). A small molecular weight protein (MW 25,000) observed in the goat FF show progressive increase in the concentration from small to large follicle. This protein has not been reported in FF from other species.

This study has clearly shown that the mean follicular fluid: serum concentration ratio of different follicular proteins is inversely correlated to the molecular size, except for minor differences which might be due to individual molecular characteristics or due to retention of glycoproteins on gels (Andrews, 1964). Thus our results strongly support the hypothesis that follicular fluid-proteins in goat are derived from serum by filtration. Our findings that the smaller proteins are found in higher concentrations and larger protein in lower concentrations in FF than in serum are also in agreement with previous findings (Shalgi *et al.*, 1973; McGaughey, 1975; Anderson *et al.*, 1976; McNatty, 1978).

The failure to detect follicle-specific protein in goat is in agreement with previous negative results with immunization experiments (Manarang-Pangan and Menge, 1971; Beck and Sheldon, 1972; Anderson *et al.*, 1976). The only protein found, which is absent from serum, is fibrinogen-like protein. There are at least two sources of protein in follicular fluid: one originates from an extra folli-

cular source (the plasma transudate) and the other from cells within the follicle. Protein derived from cell within the follicle have not been identified in follicular fluid. Failure to detect follicular specific antigens may be because of low

titres of these antigens derived from granulosa cells.

Acknowledgement

This research was supported by Indian Council of Medical Research.

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Circulating Cortisol Levels During Different Phases Of Reproduction In Goats

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ABSTRACT

The circulating levels of cortisol were monitored during oestrous cycle, early pregnancy, parturition and during post partum period in goats. The cortisol hormone levels were estimated by a direct radioimmuno assay method without extraction of plasma. The plasma cortisol levels were significantly higher ($P < 0.05$) on the day of oestrus in all goats which settled to service whereas this elevation was non-significant for non pregnant goats. Subsequently, the levels were not significantly different in pregnant and non pregnant goats during 21 days after breeding. The hormone levels fluctuated among pregnant goats non-significantly upto 145 days of gestation without showing any definite trend during this period. However, a surge ($P < 0.01$) was observed one day before and the day of parturition, which subsequently decreased significantly during early post partum period. The study provides evidence to association of elevated cortisol to the physiological event of oestrus and parturition in goats.

* * *

Advances during the past decades in reproductive endocrinology have focussed attention on the role of cortisol during various physiological events in reproduction, with the major glucocorticoid produced by adult sheep being cortisol

(Alexander *et al.*, 1972; Basset and Hinks, 1969; Blair *et al.*, 1963). Maternal plasma corticoid changes in cycling and periparturient bovines have recently been documented (Prakash, 1984). Circulatory cortisol as a measure of animals response to a stress factor or to environment has been extensively investigated among others by Rhynes and Ewing, 1973; Christinson and Johnson, 1972 in cattle; Panaratto and Ferguson 1968 and Panaratto and Vickery, 1970 in sheep. Most of the earlier studies mainly employed competitive protein binding assay technique for total corticoids. Bhattacharya *et al.*, 1980 investigated plasma corticoid using this assay estimation during oestrous cycle and pregnancy in goats, but no literature is available using sensitive assay methods regarding the circulatory plasma cortisol levels in this species during oestrous cycle, gestation and peripartum periods. This investigation was therefore undertaken to investigate the plasma profiles of this hormone and its association with the physiological events mentioned above using a sensitive radioimmunoassay technique.

Materials and Methods

Cycling crossbred goats (Saanen \times Alpine \times Beetal) were selected from the National Dairy Research Institute herd and were mated with bucks on the day

of oestrus as per their mating schedule. Blood samples for the estimation of cortisol were collected from the jugular vein of these animals on the day of oestrus (day 0) and on every alternate days upto day 25 of breeding. Following this a single sample was drawn from animals every fifteenth day upto day 120 of gestation. Animals were followed more closely by drawing samples every third day till four days before term. Daily samples were taken on day -2, -1, 0 (parturition) +1, +2, +3, +4 and again every third day during the first 22 days of post partum period. The plasma was separated and stored at -20°C pending cortisol hormone estimation.

Radioimmunoassay of cortisol hormone:

A direct radioimmunoassay technique without extraction (Radioassay systems Laboratory, California, USA), was adopted with some modifications. 25 μl of blood plasma in duplicate was used for the estimation of cortisol hormone. The cortisol binding protein was denatured by keeping the plasma samples at 98°C for 10 min in a water bath. Each assay tube in duplicate contained 25 μl of blood plasma, 3.75 ml of 0.1M PBS buffer (pH 7.0); 100 μl labelled cortisol (10,000 cpm) and 100 μl (1:20,000 dilution) specific cortisol antiserum. The tubes were vortexed and incubated for 16 hr at $+4^{\circ}\text{C}$. The bound and unbound hormone was separated using 200 μl of dextran coated charcoal suspension at 4°C . The

TABLE 1: Accuracy of the Radioimmunoassay for the Estimation of Cortisol

Replicates	Hormone added (pg)	Hormone recovered (pg)	Recovery percent
4	250	239.20 \pm 10.72	95.70
4	500	585.25 \pm 37.03	117.05
4	1000	1183.36 \pm 79.80	118.33

supernatant was decanted and counted after adding 5 ml cock tail with Liquid Scintillation Counter. The sensitivity of the assay was 8 pg/tube, the interassay and intra-assay variations were 6.5% and 3.25%, respectively. The accuracy of the assay technique is shown in Table 1.

Results and Discussion

The data on cortisol levels during oestrous cycle was classified on the basis of pregnancy status of these animals following breeding into pregnant and non pregnant groups (Table 2). The animals in both the groups showed distinct variability in the levels of cortisol on different days of oestrous cycle. The peak levels of 19.07 ng/ml were obtained on the day of oestrus in pregnant group and levels sharply and significantly ($P < 0.05$) fell by first 2 days of cycle. In this group between day 3 and 21 the levels ranged between 2.67 and 7.51 ng/ml. In the non pregnant group, the mean cortisol value on the day of oestrus,

TABLE 2: Mean Plasma Cortisol Levels (ng/ml) in Pregnant and Non-pregnant Goats During Estrous Cycle Following Breeding

Days of Breeding	Pregnant	Non-Pregnant
0	19.07 \pm 9.94(4) ^a	9.91 \pm 3.25(4) ^a
1	4.95 \pm 2.43(4) ^b	5.53 \pm 1.25(5) ^a
3	2.67 \pm 1.02(4) ^b	5.44 \pm 1.41(4) ^a
5	7.39 \pm 3.51(4) ^b	4.75 \pm 1.25(6) ^a
7	5.79 \pm 2.53(4) ^b	4.37 \pm 1.27(6) ^a
9	4.91 \pm 1.12(4) ^b	3.82 \pm 0.76(6) ^a
11	7.19 \pm 2.83(3) ^b	3.88 \pm 1.33(6) ^a
13	3.41 \pm 0.82(4) ^b	6.79 \pm 1.70(5) ^a
15	4.22 \pm 0.90(4) ^b	5.57 \pm 1.76(5) ^a
17	5.01 \pm 2.68(3) ^b	6.68 \pm 1.33(6) ^a
19	5.20 \pm 2.95(3) ^b	7.39 \pm 3.57(5) ^a
21	7.51 \pm 7.31(3) ^b	8.69 \pm 3.48(5) ^a

Mean values with the same superscript are not different from each other.

TABLE 3: Mean Plasma Cortisol (ng/ml) during Gestation Among Goats

Days of breeding	Pregnant
23	9.31±5.61(3)
25	4.99±0.40(2)
45	3.52±0.82(7)
60	4.10±0.85(3)
75	4.76±1.85(4)
90	4.18±1.44(3)
115	4.37±1.16(4)
120	3.10±0.71(5)
130	6.11±1.51(7)
133	4.39±0.54(7)
136	6.18±1.15(12)
139	3.58±0.53(6)
142	5.75±1.26(9)
145	8.17±2.30(8)

TABLE 4: Peripartum Plasma Cortisol Levels (ng/ml) in Goats

Days from parturition	Pregnant
-5	8.04±2.31(12)
-2	9.67±2.36(9)
-1	23.90±14.35(2)**
0	22.95±5.13(7)**
+1	6.75±2.13(7)
+2	10.15±2.37(8)
+3	4.86±0.68(4)
+4	5.87±2.31(4)
+7	5.70±0.80(2)
+10	3.84±2.22(3)
+13	3.47±1.84(4)
+16	6.79±1.23(4)
+19	5.28±2.19(4)
+22	4.43±3.04(3)

Day 0 refers to the day of parturition.

though highest during the cycle, was not significantly different from the values obtained on other days. Comparing the cortisol levels among pregnant and non-pregnant animals, however, no statistically significant difference was observed. The mean levels obtained during the cycle, except at oestrous, being of the same order.

After breeding, during pregnancy plasma cortisol levels fluctuated (Table

3) in the same range as described above, and the differences between the values obtained on different days were not significant. Significant increase in cortisol was obtained at the time of parturition (Table 4) when the levels showed four to five fold increase. These levels were of a much higher magnitude than those observed during oestrus. The elevated peak phase lasted for a short period and by day one post partum the mean levels had declined to 6.74 ng/ml from the level of 22.95 ng/ml observed on the day of parturition. Post partum levels upto day 22 showed no significant differences with mean levels fluctuating between 10.15 and 4.43 ng/ml.

The pattern of peripheral concentration of cortisol as described above indicates a physiological elevation of cortisol incident to oestrus. This elevation has also been reported among cattle (Adeyemo *et al.*, 1981), among buffaloes (Rao and Pandey, 1981) and in earlier study in goats (Bhattacharya *et al.*, 1980). In contradiction to these observation Russel *et al.* (1983) reports a very low level on the day of oestrus and higher levels on day 6 and 9 of the cycle after breeding. Whereas no change in the glucocorticoid level at any stage of oestrous cycle have also been reported among cows (Echternkamp and Hansel, 1973). The discrepancies in the magnitude of cortisol fluctuations obtained in these studies could be attributed to the different assay techniques. persuing the data for individual animals, cortisol elevation of a lower and higher magnitude were evident incident to behavioral symptoms. The symptoms were related to the magnitude of circulating plasma. The higher magnitude of cortisol at oestrus can be due to either an increase physical activity associated with oestral behaviour or due

to activation of a hypothalamo-hypophyseal adrenal axis. High circulating oestrogens stimulated the release of AC-TH (Gemzell, 1942), may also be the mechanism involved as oestrogen level peak around oestrus.

The rise in circulating cortisol at parturition was possibly as a result of rather than the cause, of parturition and was stress induced response. Since under dexamethasone induced parturition no increase in cortisol is observable. The physiological significance of sudden increase in cortisol level at parturition

in goats is not well documented because of the lack of systemic fetomaternal studies. However, the surge in cortisol levels around parturition may indicate adrenal association with maternal milk secretion (Convey, 1974) parturition (Prasad and Madan, 1984) or increase in abdominal contractions (Schwalm and Tucker, 1978).

Acknowledgement

The authors are grateful to the Director, National Dairy Research Institute, Karnal for providing the facilities for this investigation.

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Acid Base And Blood Gas Changes In The Foetal Kid And Amniotic And Allantoic Fluids

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ABSTRACT

Studies were conducted in 12 adult goats at 1-5 months of gestation to investigate the blood gas changes in the foetal and dam blood. The pH and carbon dioxide tension of amniotic and allantoic fluids were also investigated. The study showed that the foetal blood remains acidotic in comparison to dam blood at all stages of gestation, with high PCO_2 values. The bicarbonate values of the foetal blood were much lower than the dam blood during 1-2 months of gestation. Both amniotic and allantoic fluid were more acidic in comparison to foetal and maternal blood, particularly at late stages of gestation.

* * *

The changes in the circulation of the foetus and its environment has been the subject of many investigations in the past. A number of studies have been conducted to investigate the acid base and blood gas changes that occur in the foetus at various stages of gestation and immediately after delivery. Such studies have been conducted in Sheep (Asali *et al.*, 1963; Curet, 1970 and Comline and Silver, 1970a) and dairy cows (Wilson *et al.*, 1977). Few studies are also available on certain aspects in mare (Comline and Silver, 1970b) and in goats (Meschia *et al.*, 1965). The present study was conducted in pregnant goats at various

stages of gestation to study the changes in the acid base and blood gases of the foetal blood, and allantoic and amniotic fluids.

Materials and Method

Twelve adult goats, 2 to 3 years of age and at various stages of gestation were used in this study. The pregnancy was confirmed by manual palpation. These animals were comfortably controlled in lateral recumbency and routine caesariotomy, under aseptic precautions, was done to exteriorize the foetus; under local infiltration analgesia. Immediately after, a careful incision over the uterine wall, the amniotic and allantoic sacs were allowed to emerge. Amniotic and allantoic fluids were collected anaerobically, separately, in disposable syringes for their gas measurements. Subsequently, the foetus was exteriorized with minimal manipulation and damage to the placenta and attachments. Minute careful dissection was performed to separate the umbilical arteries and veins, and blood samples collected immediately under anaerobic conditions for blood gas measurements. The maternal venous blood was collected immediately before operation from the jugular vein with minimal disturbance to the animal.

After the delivery of the foetus and collection of blood samples, the umbilical

TABLE 1: The Acid Base Status and Blood Gases of the Maternal Venous Blood at Various Stages of Gestation.

Stage of gestation months	1-2 months	2-3 months	3-4 months	4-5 months
pH	7.341 ± 0.035	7.319 ± 0.029	7.297 ± 0.050	7.333 ± 0.016
pCO ₂	33.5 ± 3.85	36.8 ± 1.65	34.5 ± 5.69	32.1 ± 2.76
pO ₂	62.8 ± 7.41	42.8 ± 0.58	38.8 ± 2.40	51.7 ± 1.04
HCO ₃	17.6 ± 0.38	18.5 ± 0.52	16.3 ± 2.62	16.8 ± 1.76

TABLE 2: The Acid Base Status and Blood Gases of the Foetal Venous Blood at Various Stages of Gestation

States of gestation months	1-2 months	2-3 months	3-4 months	4-5 months
pH	7.183 ± 0.041	7.197 ± 0.084	7.318 ± 0.016	7.159 ± 0.099
pCO ₂	27.2 ± 4.49	52.5 ± 12.1	33.5 ± 1.16	48.96 ± 8.58
pO ₂	49.7 ± 2.87	52.05 ± 12.5	36.6 ± 0.71	41.1 ± 3.19
HCO ₃	9.8 ± 1.38	19.30 ± 1.59	17.08 ± 0.84	16.10 ± 1.08

vessels were severed, ligated and the abdominal wound of the dam was closed routinely.

The gestation period was categorised after measurements of the Crown-Rump length of the foetus, as between 1-2 months, 2-3 months, 3-4 months and 4-5

TABLE 3: The Acid Base Status and Blood Gases of the Foetal Arterial Blood at Various Stages of Gestation

State of gestation parameters	1-2 months	2-3 months	3-4 months	4-5 months
pH	7.075 ± 0.010	7.181 ± 0.065	7.243 ± 0.024	7.074 ± 0.077
pCO ₂	45.5 ± 4.01	54.02 ± 10.07	38.73 ± 2.54	67.6 ± 9.17
pO ₂	33.65 ± 1.96	28.93 ± 2.16	22.33 ± 0.97	27.42 ± 1.94
HCO ₃	12.85 ± 1.65	19.17 ± 0.31	16.22 ± 1.27	18.40 ± 1.11

TABLE 4: The pH and pCO₂ Values of Allantoic and Amniotic Fluids During Different Stages of Gestation

State of gestation Parameters	1-2 months	2-3 months	3-4 months	4-5 months
ALLANTOIC FLUID				
pH	7.320 ± 0.071	N.D.	6.628 ± 0.111	6.833 ± 0.026
pCO ₂	27.8 ± 2.27	N.D.	36.5 ± 0.89	41.87 ± 2.03
AMNIOTIC FLUID				
pH	7.253 ± 0.098	N.D.	6.860 ± 0.38	7.063 ± 0.97
pCO ₂	30.76 ± 0.66	N.D.	32.97 ± 5.04	35.63 ± 2.79

ND = Not Detectable

months. All the data related to foetus was plotted accordingly.

The gas analysis of the fluids and maternal and foetal blood was done on a gas analyser* at 37°C under anaerobic conditions. The bicarbonate of the mater-

* Radiometer Copenhagen, The B.M.E 33, microequipment

nal and foetal blood was calculated according to Henderson Hassalbalch equation using measured pH and $p\text{CO}_2$.

Results

The mean with S.E. of various parameters relating to the venous blood of the dam are shown in Table-1. The data on foetal venous and arterial blood are shown in Tables 2 and 3, respectively. The data shows that the foetal arterial blood has lower pH and $p\text{O}_2$ values and high $p\text{CO}_2$ values in comparison to the venous blood of the dam, at all stages of gestation. The foetal arterial bicarbonate values during 1-2 months of gestation are lower in comparison to the venous blood of the dam. At later stages the bicarbonate values in both are comparable. The foetal venous blood shows higher acidity because of high $p\text{CO}_2$ values.

The pH and $p\text{CO}_2$ values of amniotic and allantoic fluids are shown in Table-4. The data shows that with the advancement of gestation the allantoic and amniotic fluids have lower pH values in comparison to the venous blood of the dam.

Discussion

The results of the present study show that the foetus in goats remains in a state of acidosis throughout the gestation period in comparison to the dam. This acidosis appears to be related to the high $p\text{CO}_2$ values as is clear from the data shown in Table-3. The results are in agreement with the findings of Kaiser and Cermmings (1957) and Assali *et al.* (1963) in sheep. It has also been suggested that this low pH of the foetal blood could be related to metabolic acidosis (Rooth and Sjostedt, 1962). In the present study

low bicarbonate levels were evident in the foetal venous and arterial blood during 1-2 months of gestation. However, subsequently the bicarbonate values of the foetal blood were comparable to that of the dam. Therefore, during 1-2 months of gestation both foetal hypoxia and metabolic acidosis were possibly responsible for low pH of the foetal blood. It should however be noted that the studies on the foetal blood in the present investigation were carried out after the foetus had been exteriorized from the uterus. Therefore, it is possible that this procedure along with any possible damage to the placenta might have affected the transfer of hydrogen ions and carbon dioxide from the foetus to the mother. It has been shown previously that under such circumstances the foetal hypoxia may get aggravated and that this aggravation might not be related to the blood $p\text{CO}_2$ values (Assali *et al.*, 1963). It has also been suggested, previously, that this foetal acidosis may not have any pathological significance (Kaiser 1959) because it is possible that the foetal blood appears acidotic because it is compared to adult standards (Assali *et al.*, 1963).

The pH of both amniotic and allantoic fluids in the present study was lower than the foetal and maternal blood, particularly after three months of gestation. The data in Table-4 shows that higher acidity of the allantoic fluid during later stages of gestation could be related to the higher $p\text{CO}_2$ values; while for amniotic fluid the increase in the $p\text{CO}_2$ values at the same stages was only marginal. It, therefore, appears that the transfer of hydrogen ions and carbon dioxide to these fluids could be responsible for higher acidity. The pH of both amniotic and allantoic fluids in the present study were comparable to those reported by Assali

et al. (1968) in sheep but the $p\text{CO}_2$ values were comparatively lower. During 4-5 months of gestation the amniotic fluid was more alkaline in comparison to allantoic fluid which is in agreement with the findings of Mellor and Slater (1971)

in ewes.

The results of the present study reveal that in foetal kids the blood pH is acidotic in comparison to dam but the relative contribution of metabolic and respiratory components for the same remains unclear.

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Estrus Synchronization And Fertility In Adult Cycling Ewes Using Prostaglandin F₂ Alpha And Progesterone

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ABSTRACT

Oestrus synchronisation and fertility was studied in adult cycling Nali ewes using Prostaglandin F₂ alpha (PGF₂ alpha) 15 mg. I/M per ewe on Day 9 of oestrus, Progesterone 25 mg. I/M on Day 0 (day of treatment), 7 and 14 of treatment irrespective of day of oestrus. Group 2 (PGF₂ alpha) comprised 20 ewes, group 3 (Progesterone) 16 ewes, and group 1 (control) 4 ewes. Synchronisation of oestrus was 100% in group 2 and 68.75% in group 3 whereas no synchronisation was observed in group 1. Interval of onset of oestrus from start of treatment, oestrus duration and cycle length were reduced in prostaglandin treated group compared to progesterone treatment and control. All the ewes laparotomised under prostaglandin treatment ovulated although only 88.8% in progesterone treatment had ovulations. Time required for ovulation from onset of oestrus was about 2 hrs. less under prostaglandin treatment than progesterone treatment. Lambing percentage was 81.25% under prostaglandin treatment, 66.6% under control and only 16.6% under progesterone treatment. Prostaglandin treatment appeared to be most efficacious for oestrus synchronisation and fertility.

* * *

Prostaglandin F₂ alpha (PGF₂ alpha) has been reported to cause functional and structural luteolysis in sheep (Umo, 1975). The level of this luteolytic agent has been found to increase towards the end of oestrus cycle in the uterine venous plasma of sheep (Thorburn *et al.*, 1972). Effective synchronisation of oestrus has been achieved in ewes using Cloprostenol and PGF₂ alpha (Dobson and Ward, 1977). Oestrus synchronisation in ewes with progesterone administration through systemic route as well as vaginal pessaries have been reported by various workers (Davies, 1960).

The present investigation was undertaken in adult cycling ewes of Nali breed to observe the reproductive responses with particular reference to oestrus synchronisation, ovulation and fertility upon treatment with PGF₂ alpha, Progesterone and Colostrum.

Materials and Methods

Selection of animals and hormone administration:

Twenty-four healthy cyclic ewes of Nali breed were selected on the basis of previous oestrus detection record, out of which 20 were assigned to experimental group 2 and the rest 4 served as control (Group 1). After ascertaining day 9 of oestrous cycle of each individual ewe,

TABLE 1. Oestrus synchronization and behaviour in adult ewes under different treatments.

Group	No. of ewes	Ewes showing synchronized oestrus No.	Interval of onset of oestrus from treatments (hr)	Oestrus duration (hr)	Cycle length (d)
1	4	—	222.00±2.00 ^a	33.33±0.67	18.33±0.33 ^a
2	20	20 (100)	49.00±0.96 ^c	25.50±1.83	12.92±0.62 ^b
3	16	11 (68.75)	93.00±5.84 ^b	26.63±3.24	18.58±1.29 ^a

Note: 1. Values with same superscripts within each parameter did not differ significantly from each other.
2. Values under parentheses indicate percentage.

the animals were administered PGF₂ alpha (Lot 40, 213-1 Upjohn International Inc.) at the rate of 3 ml. per ewe (containing 5 mg. dinoprest per ml.). Group 3 comprised of 16 ewes and each was injected 25 mg Progesterone (Prognon (4)-dion-beta 20) dissolved in propylene glycol I/M on day 0 (day of treatment), 7 and 14.

TABLE 2. Induced ovulatory responses observed on laparotomy in adult ewes

Group	No. of ewes laparotomized	No. of ovulation detected		Ovulation percentage
		Right ovary	Left ovary	
1	1	—	—	—
2	4	3	1	100
3	9	4	4	88.8

Animal observations:

Oestrus detection through teaser ram was done 12 hourly daily followed by 4 hourly from second day of treatment until end of oestrus. Ewes were subjected to laparotomy following normal procedures, 24 to 36 hr. after onset of oestrus to determine the time of ovulation.

Results and Discussion

Oestrus behaviour and ovarian activity:

The results presented in Tables 1 and 2

showed that PGF₂ alpha was fully effective in inducing synchronisation of oestrus followed by ovulation. Progesterone was nearly 70% effective but colostrum treatment did not show a positive response. The time interval between PGF₂ alpha administration and onset of oestrus was 49 hr. In progesterone treatment group the time interval between withdrawal of treatment and oestrus synchronisation was 93 hr. The differences were found significant between all the treatments and control groups. Oestrus duration was apparently reduced (though statistically not significant) under PGF₂ alpha and progesterone treatments in comparison to control.

The mean cycle length following PGF₂ alpha treatment (Table 2) was significantly reduced by about 6 days in comparison to other two treatments. This was expected since it is known to exert a potent luteolytic effect.

Total and complete synchronisation of oestrus in group 2 showed that PGF₂ alpha is effective in inducing behavioural expression of oestrus and luteolysis when given through systemic route during mid-luteal phase of the cycle. A single I/M injection of 15 mg. PGF₂ alpha on day 8 was found by Hawk (1973) to be effective in inducing oestrus

synchronization in 45 out of 52 ewes (86.5%). The interval of onset of oestrus was 2 to 3 days following PGF₂ alpha injection, which compares well with the observations in the present study. Using synthetic analogues of PGF₂ alpha (Cloprostenol), Trounson *et al.*, (1976) demonstrated that oestrus was synchronised in 84% Welsh Mountain ewes within 29-48 hr. of treatment, whereas in the present study the corresponding values were 100% and 43-59 hr., respectively, which shows better response of the indigenous Nali breed to PGF₂ alpha treatment.

All the ewes under the treatment exhibited intense signs of oestrus with full receptivity to rams which indicated that PGF₂ alpha did not interfere with the normal physiological functioning at hypothalamo-hypophyseal axis nor was there any side effect during the whole experimental period or thereafter. Significant reduction in cycle length in group 2 (Table 1) indicated premature luteo-

lysis due to the effect of PGF₂ alpha. Similar observations have been made by Douglas and Ginther (1973) about shortening of oestrous cycle following PGF₂ alpha treatment. In case of group 3, receiving progesterone treatment, although the response was not poor, yet the treatment leading to oestrus synchronisation did not present a highly satisfactory picture like in group 2. A good degree of inhibition of oestrus with three dosage schedules of progesterone was not fully achieved as only about 70% of the ewes were closely synchronised at the end of second dose treatment. Those ewes which did not exhibit oestrus after second progesterone injection were given the last dose of progesterone on day 14. But oestrus was not synchronised in them which seemed to be on account of individual variation in response to the blocking effect of progesterone within the same breed. The cycle length in the progesterone treated group (group 3) was unaffected and compared closely

TABLE 3. Probable time of ovulation based on 4 hourly oestrus detection and laparotomy in ewes under different treatments.

Description	Treatment groups		
	1	2	3
No. of ewes under observation	1	4	8*
Ovulation time from onset of oestrus (hr.)	28.0	28.5±0.5	30.7±3.9
Ovulation time from beginning of treatment (hr)	75.0	76.5±1.0	133.6±5.2

* One animal ovulated without signs of overt oestrus.

TABLE 4. Conception and lambing behaviour in adult ewes

Group	No. of ewes	Concieved at				Lambing	
		1st oestrus		2nd oestrus		No.	%
		No.	%	No.	%		
1	3	2	66.6	—	—	2	66.6
2	16	11	68.75	4	25	13	81.25
3	12	2	16.6	—	—	2	16.6

with the control (Table 1), which indicated no adverse effect of the hormone on the normal physiological rhythm of ovarian activity. The average ovulation time from the onset of oestrus was 28.5 hr. in PGF₂ alpha and 39.7 ± 3.9 hr. in progesterone treated groups (Table 3). The corresponding value for normal control was 28.0 hr. It thus appeared that PGF₂ alpha does not interfere with the normal pattern of ovulation, whereas progesterone treatment delays ovulation presumably because of higher titre of progesterone in the blood.

Conception & lambing pattern:

Result of induced breeding behaviour is presented in Table 4. In the PGF₂ alpha treated group, of all the ewes mated to rams, only 68.75% conceived at the synchronised oestrus while rest 25% settled at the second oestrus. One ewe did not settle. This indicated that PGF₂ alpha had smooth sailing with normal physiological function of the reproductive tract both in implantation

and growth of embryo and survival of foetus till termination of pregnancy. Of course, there was one ewe which gave birth to twins while another one suffered from dystokia. The foetus in this case was normal but could not be delivered and succumbed during parturition. All other lambings were normal. Recent report of Allison and Kelly (1978) showed that oestrus in ewes, treated with 2 injections of Prostaglandin (ICI 30996) ten days apart, half of which also received PMSG 750 IU 2 days before administration of prostaglandin, was effectively synchronised but fertility at the first oestrus after the end of treatment was low. In case of progesterone treated ewes, the unexpectedly poor fertility may be conceived in the light of reports by many workers (Davies, 1960; Hogue *et al.*, 1962; Foote and Waite, 1965; Hawk and Conley, 1971; Robinson, 1971) who indicated that progesterone treatment was coming in the way of effective sperm transport in the female genital tract.

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Comparative Appraisal Of Various Acrosomal Integrity Tests

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ABSTRACT

The acrosome of the sperm cell literally spearheads the process of fertilization. Therefore, it is not surprising that acrosomal studies attracted the attention of many researchers. After the advent of electron microscopy, the ultrastructural architecture of this organelle is better understood and the damage caused by the various treatments or various hereditary conditions was appraised more critically.

During the past decade, the zonolytic activity of acrosin was studied more intensely by employing the immunofluorescent methods. Although these results are more informative, are expensive and demand the use of delicate and sophisticated techniques.

This paper attempts to describe the relative merits, demerits, validity and applicability of various tests developed to appraise the acrosomal integrity.

* * *

The reasons for the reproductive failures caused by the male source are manifold and complex. The acrosome of the sperm literally spearheads and breakthrough the cumulus oophorus cells (by hyaluronidase activity), the Corona radiata (by corona penetrating enzyme) and ultimately the zona pellucida of the ovum (by acrosin) (Zaneveld & Polakoski, 1976) to initiate fertilization

and start a new life. The sperm cell is about 80,000 times smaller (Asimov, 1962) than the ovum and thus it is more agile to move in the uterine environment. Yet, the sperm has to undergo capacitation in the female genital tract and acrosomal reaction in the vicinity of the ovum and release the enzymes sequentially to initiate fertilization. The acrosomal enzymes or substance are described as lipoglycoprotein complexes (Mann, 1969).

Besides the usual acrosomal abnormalities, the acrosomal abnormalities of hereditary nature were also described by Hancock (1953) and Donald and Hancock (1953) in bulls. Bane (1961) and Bane & Nicander (1966) described the abnormality in boars.

It is now accepted that intact acrosomal cap is an essential prerequisite for fertilization.

Of the three sperm abnormalities *viz.*, head, middle piece and tail abnormalities, the middle piece abnormalities were considered to be serious because the energy required for the propulsion is generated in the mitochondrial helix of the midpiece. But after reaching the proximity of the egg, the acrosomal enzymes assume importance in dispersing the surrounding cells, penetrating the zona pellucida and in actually entering the perivitelline space. With the increasing realization of acrosomal importance

several tests were developed to score the intact acrosomes, in neat, diluted, preserved, deep frozen and thawed semen.

Acrosomal Integrity Tests

The conventional vital staining methods used for live and dead counts and for scoring the other abnormalities, were found to be somewhat inadequate as the live spermatozoa remain unstained by eosin and lend no appreciable colour contrast required for critical appraisal of acrosomal cap under the ordinary light microscope.

Naturally spermatologists started looking for dyes or dye combinations other than the routine eosin-nigrosin or aniline blue stains.

1. *William's stain*: The stain was recommended by Williams (1920) for studying the sperm abnormalities. Pretreatment with chloramine solution is used for removing the obscuring seminal proteins. This stain is still favoured by Swedish researchers.

2. *Rose Bengal and Crystal violet*: Rose Bengal staining was used by earlier researchers (Herman & Swanson, 1941). The stain has the additional advantage of staining and fixing simultaneously because of the formalin component. The stained slides are convenient for the rapid diagnosis of oligospermia or azoospermia.

The crystal violet and Rose Bengal combination introduced by Williams (1937) and Williams *et al.* (1934) was more useful for the acrosomal studies, as the Rose Bengal component, stains the membranous cap pink and the crystal violet component, stains the underlying nucleus blue. Further, the Rose Bengal does not stain the mucus and the removal of the mucus with chlorozane solution is not necessary.

3. *Periodic acid Schiff (PAS) stain or Feulgen's stain*: The carbohydrate moiety of acrosomal complex was well recognized and advantageously used for staining the acrosome. Mann (1969) reported the occurrence of the five carbohydrate components *viz.*, sialic acid, fucose, galactose, mannose and hexosamine. He suggested that these are structural components of the acrosome rather than active participants in metabolic process.

Since the acrosomal system forms from the Golgi apparatus these structural changes are also used as markers in the evaluation of the spermiogenic defects at spermatid maturation stages. Bull testicular tissue sections were stained with PAS method for diagnosing the acrosomal cap abnormalities of spermatids (Saacke *et al.*, 1968). Feulgen's and light green (Counterstain) were used for demonstrating the acrosomal cap (Bloom & Fawcett, 1976). This differential staining method is usually preferred for studying the acrosomal cap of the spermatids during spermiogenesis from tissue sections rather than the ejaculated sperm.

4. *Heidenhain's iron haematoxylin*: This method was tried by Hancock (1946) and is not used any more as the appraisal of acrosomal damage is somewhat subjective, depending on the depth of the staining.

5. *Casarett's stain*: This is another differential staining technique introduced by Casarett (1953) for studying the various regions of the sperm cell (including acrosome). This method has not become popular for studying bull spermatozoa.

6. *India ink preparations*: Blom (1977) recommended the use of good quality India ink smears for appraising the acrosomal denudation in the bovine sperm

samples. Prior fixation is not required. In addition, the smears can be kept for future reference. This method is rapid and cheap and can be used routinely.

7. *Eosin B and Fast green*: Wells and Awa (1970) used this staining technique for studying the acrosomal characteristics.

8. *Acridine orange*: The Lysosomal nature of acrosomal system is advantageously used for studying the acrosomal caps of rats and guinea pigs (Allison, 1967). However, the application of fluorescent dyes, for studying the bovine spermatozoa is not popular. This may be due to non-availability of the fluorescence microscopic facilities for routine use.

9. *Giemsa stain*: The stain was introduced by Hancock (1953) for studying the acrosomes. Giemsa stain is known to be sensitive to the change in the H-ion concentration. Therefore, the stain should be properly buffered before the application. Different brands of Giemsa are also used (BDH, Gurr, etc.). Saacke *et al.* (1968) and Hrudka (Watson, 1975) used the Giemsa with modifications. Saacke *et al.* (1968) classified acrosomal abnormalities into four categories, viz., knobbed, incomplete, ruffled and abnormal nuclear cap.

Besides, the stainability is affected by the presence of egg-yolk and glycerol components of the conventional extenders. In the absence of better alternative staining procedures, Giemsa is still preferred with its attendant disadvantages.

10. *Other differential staining techniques*: The quest for studying the parts of the sperm is still on, especially those spermatozoa necessarily subjected to various treatments such as, extenders, storage at ultra low temperatures for different periods and freeze-thawing. Chacarov and Mollova (1976) tried

different dyes supplied by I.C.I. (Imperial Chemical Industries, Manchester, England) for staining the neat and deep frozen (-196°C) sperm. The combination of Procion Printing Green B and Procion Brilliant Red M8BS gave excellent results. The acrosome was especially discernible even in the deep frozen samples.

At present, we (Benjamin *et al.*, 1983) are trying Procion Tarquoise and Procion Red (Printing Green is no longer manufactured). The results obtained so far are encouraging and the acrosomal cap stains bluish green, nucleus stains reddish and cytoplasmic droplet stains greenish. However, the stain is fading away on keeping. Some more trials are to be conducted on buffalo semen preserved in various popular extenders. Attempts are being made to standardise this method.

11. *Differential Interference microscopic and Phase contrast microscopic studies*: The unfixed and unstained semen smears can be studied in "Life-like-manner" (without various processing artifacts). If a biotherm is attached to the stage (maintained at 37°C) even motility (mass or individual) can be graded from fresh preparations (droplets). Saacke & White (1972) established a positive correlation between the percentage of intact acrosomal caps (after post-thawing incubation at 37°C). This correlation (r) was closer than those obtained between fertility and motility or abnormal sperm counts. They further described the apical ridge on the anterior most portion of the sperm head; The apical ridge was observed under the differential contrast interference microscopic examination. Since the acrosomal membranes are more sensitive to the osmotic changes, than the post-nuclear cap membranes, they

concluded that the degree of obliteration (due to swelling or bursting) of apical ridge can be used as an indicator of the acrosomal damage caused by cryoinjury due to freezethawing or the storage method adapted (Saacke & Marshall, 1968).

12. Nowadays, Phase contrast microscopy is commonly used in conjunction with biotherm (warm stage) in well-equipped semen laboratories. Furthermore, the desired contrast i.e., object bright, and back-ground dark, or the reverse can be achieved by using the appropriate phase plates. These recent innovations enable one, to make critical and prolonged examination of live (unfixed/unstained) sperm. Aamdal (1951) examined the spermatozoa under phase contrast microscope after staining with opal blue, and he described an acrosomal abnormality, termed as eversion of galea capitis.

13. *Electron microscopy (E/M)*: Although E/M studies are exorbitantly expensive for routine work, they are extremely useful academically. The E/M studies on appraising sperm abnormalities especially the acrosomal conditions in health and in disease conditions (inherited also) or under deep frozen conditions are invaluable to researchers working on spermatology (Saacke & Almquist, 1964; Saacke & Marshall, 1968; Saacke, 1970; Saacke & White, 1972; Fawcett, 1975; Larson *et al.*, 1976; Settergren & Nicander, 1968; Hrudka, 1983). The aforesaid ultrastructural studies removed the former confusing terms such as galea capitis etc. The capacitation and the acrosomal reactions are better understood. At IVRI we used E/M studies for assessing the ageing effects in extenders (Tupol-preserved semen). The condition of apical ridge forms another useful guide-

line; if E/M studies and differential contrast interference studies are combined (Saacke & Marshall, 1968; Saacke, 1970; Saacke & White, 1972).

14. *Fluorescent Antibody technique (FAT)*: After the purification of acrosin, the antibodies are produced in rabbits and labelled with a fluorescent dye (FITC, Fluorescein isothiocyanate). These labelled antibodies are used for staining the acrosomal regions (under test) precisely. The acrosin is (acrosomal protease) known to possess zonolytic activity. Acrosin is associated with both the outer and inner acrosomal membranes. While the presence of acrosin on the inner membrane confirms the zonolytic activity and the presence of acrosin on the outer membrane, leaves open the possibility that acrosin has some activity other than the zonolytic activity (Johnson *et al.*, 1981). Johnson *et al.* (1976) isolated and purified the porcine acrosin. Garner & Easton (1977) described immunofluorescent methods for locating the mammalian acrosin. Garner *et al.* (1978) postulated that the egg-yolk of the diluents could interfere with this staining method. However, Garner (1978) visualized the possibility of biochemically assaying the fertilizing ability of a bull. But this method, demands the use of purified biochemicals, interference microscope and fluorescence microscope (fitted with fluoremetric device) for successful application. Undoubtedly this method of acrosomal integrity evaluation is very sensitive and specific and therefore necessitates the use of sophisticated immunological and biochemical assaying laboratories, manned by an excellent team of researchers.

Now, it is unequivocally realized that intact acrosomal cap is an essential

prerequisite for the fertilization. A reliable test designed to determine the acrosomal integrity (under natural or experimental conditions) should be one of the semen evaluation criteria. Until

an economic and efficient test is developed, Giemsa staining (with minor modifications) will remain as dependable test for appraising the acrosomal integrity.

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Cryopreservation Of Buffalo Semen In Straws—Protective Action Of Sugars

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ABSTRACT

Cryoprotective properties of sucrose-mannose, lactose-fructose in freezing of Surti buffalo semen were investigated. Fructose or mannose, a monosaccharide and a permiable sugar, did not provide adequate cryoprotection to spermatozoa in absence of glycerol and buffer. Similarly lactose or sucrose, a disaccharide and a non permiable sugar did not provide adequate cryoprotection in absence of glycerol or buffer during freezing.

* * *

In bovines, in vitro preservation of semen has been extensively studied. Review of the available literature reveals that more work has been done on *Bos taurus* semen then on *Bos bubalis*. The usage of cattle semen extenders for buffalo semen has been reported by various workers resulting in significant variation in spermatozoal survival.

Glycerol has been extensively used as

cryoprotective agent for freezing of cattle and buffalo semen in ampules, pellets and straws. It has been reported that despite the presence of glycerol there is a reduction in the post thaw motility of spermatozoa in different extenders. Hence, in the present investigation cryoprotective sugars like sucrose, mannose, lactose and fructose alone or in combination were tried without glycerol and a buffer.

Materials and Methods

The present investigation was carried out on Surti bulls maintained at All India Co-ordinated Research Project on Buffaloes, Dharwar.

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

TABLE 1: Composition of the experimental extenders.

Ingredient (in parts per 100)	Extender									
	A	B	C	D	E	F	G	H	I	J
Sucrose solution	80	60	40	20	—	—	—	—	—	—
Mannose solution	—	20	40	60	80	—	—	—	—	—
Lactose solution	—	—	—	—	—	80	60	40	20	—
Fructose solution	—	—	—	—	—	—	20	40	60	80
Egg Yolk	20	20	20	20	20	20	20	20	20	20

Sugar solutions of 225 mM osmolarity were prepared and the composition of different experimental extenders are given in Table 1. Tris citric acid fructose egg yolk glycerol extender (Vasanth, 1978) which is routinely used in the buffalo breeding project 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 million live spermatozoa. Straws were filled with extended semen at room temperature and sealed with polyvinyl alcohol (PVA) powder. Identification of straws with different extenders was done utilizing different color combinations of straw and PVA powder. After 4 hours of equilibration period the straws were frozen over liquid nitrogen vapour for 10 minutes in Minnesota Valley Engineering Biological freezer and then plunged into liquid nitrogen. The straws were collected from the freezer and stored over frozen semen containers. The initial motility (immediately after extension of semen), prefreeze motility (after 4 hours of equilibration) and post thaw motility (after 2–4 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 trisodium citrate solution (3 per cent) adjusted to pH 7 with citric acid to semen samples as described by Flukes *et al.*, (1977). The percentage values of motility was transformed to arcsine values and subjected for analysis of variance. The mean comparison was done by students test (Snedecor and Cochran, 1967).

Results

The mean initial, prefreeze and post thaw motility are presented in Table 2.

The initial motility, and prefreeze motility observed in different extenders were lower than control. Sucrose or lactose alone in semen extender gave significantly ($p \leq 0.05$) higher initial and prefreeze motility than mannose or fructose alone. Gradual decrease in initial and prefreeze motility was noted as sucrose or lactose content decreased with an increase in mannose content in respective combinations. Post thaw motility observed in various combinations of extender was lower than the control. Further, with 180 and 225 mM of mannose or fructose the motility was nil.

Discussion

In buffaloes, there is paucity of information on sugar extenders without buffer

TABLE 2: The mean percentage value of initial, prefreeze and post thaw motility in sugar extenders.

Extender	Initial motility	Prefreeze motility	Post thaw motility
A	67.50 ^{bc} ± 1.53	65.00 ^d ± 2.11	5.50 ± 1.38 ^a
F	69.00 ^{yz} ± 1.48	64.00 ^y ± 1.79	8.50 ± 1.67 ^a
B	68.00 ^e ± 1.53	64.00 ^d ± 2.67	6.50 ± 1.83 ^a
G	68.33 ^y ± 1.67	65.00 ^{yz} ± 2.11	8.00 ± 1.33 ^a
C	60.00 ^b ± 3.16	53.00 ^e ± 2.81	2.00 ± 0.82 ^a
H	68.00 ^y ± 1.60	56.00 ^y ± 2.96	2.00 ± 0.82 ^a
D	29.50 ^a ± 3.02	23.00 ^b ± 2.90	—
I	67.66 ^x ± 1.53	33.50 ^x ± 3.08	—
E	23.00 ^a ± 3.67	16.00 ^a ± 2.87	—
J	36.50 ^w ± 2.36	22.50 ^w ± 3.09	—
Tris extender (control)	74.00 ^z ± 1.79	70.00 ^{dz} ± 1.82	41.00 ^b ± 3.71

Between the extenders, means with common superscripts do not vary significantly at $p \leq 0.05$.

and glycerol during semen freezing in straws. Bull semen extended with equal volume of lactose extender containing 0, 60, 120, and 180 mM of lactose without glycerol gave 0, 0, 5 and 16 per cent post thaw motility respectively and with fructose extender containing 0, 60, 120 and 180 mM of fructose without glycerol gave 0, 0, 1 and 1 per cent post thaw motility respectively for semen frozen in straws. Further, fructose became toxic at higher concentration for bull spermatozoa during freezing (Unal *et al.*, 1978). This indicated that either lactose or fructose alone at 0, 60, 120 and 180 mM levels did not provide cryoprotection to bull spermatozoa. The results in the experiment concures with the findings of Unal *et al.* (1978).

In the present study, all the four investigated sugars provided no appreciable cryoprotection to buffalo semen during freezing. However, semen extenders with sucrose or lactose alone (a Disaccharide) had slightly higher post thaw motility than fructose or mannose alone. The di and trisaccharides like lactose, fructose and raffinose provide better cryoprotection than monosaccharides like glucose, galactose fructose and as the molecular weight of the sugar

increases the cryoprotection afforded by it also increases (Salisbury *et al.*, 1978).

Buffers are known to protect bovine spermatozoa during freeze thaw cycle (Davis *et al.*, 1963; Steinbach and Foote 1964a; Steinbach and Foote, 1964b). In pellet freezing of bull semen, sugars are believed to replace the buffering salts in semen extenders (Nagase *et al.*, 1964a) and provide cryoprotection for all (Berndtson and Foote, 1972) or part of the replaced glycerol (Nagase *et al.*, 1964b). The protection afforded by large sugar molecules is different from that provided by glycerol and the two are not inter-changeable. Further, glycerol is indispensable and sugars are sometimes complimentary and useful for cryoprotection of spermatozoa. (Salisbury *et al.*, 1978).

It could be concluded that a permiable sugar like fructose or mannose (monosaccharides) and a non permiable sugar like lactose or sucrose (disaccharides) either alone or in combination do not provide adequate cryoprotection in absence of glycerol and a buffer during freezing of buffalo semen in 0.5 ml French straws.

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Studies on Characteristics Of Crossbred Bull Semen And Its Keeping Quality

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ABSTRACT

A total of 274 ejaculates from three Holstein Friesian X Sahiwal crossbred bulls were obtained to study the semen characteristic over a period of 18 months. The mean values of different semen characteristics obtained in the present investigation were, volume: 6.0 ml per ejaculate, sperm concentration: 1148 millions per ml, initial motility: 78 percent, and the percentage of live sperms (based on live and dead staining technique) was 78 percent. The average conception rate, using the semen from these bulls was 37 percent ($n=398$). At refrigeration temperature the keeping quality was found to be low with rapid deterioration. It was also observed that the freezability was poor and discard rate of freeze-thawed semen was high.

* * *

Reports on the quality of semen donated by the crossbred bulls do not satisfy the criteria suggested for normal semen (Rao *et al.*, 1979; Raja and Rao 1983). Mathew (1976) observed that the sub-standard semen obtained from these bulls lead to higher rates of elimination in selecting crossbred breeding bulls. The present investigation reports the findings of the semen characteristics, keeping quality and fertility rates obtained by using the semen of these bulls.

Materials and Methods

Three crossbred (Holstein Friesian × Sahiwal) bulls with an average age of 23 months maintained under the farm conditions of the institute, were used for the present experiment. Semen was collected regularly from the bulls at an interval of four to five days by using artificial vagina. A total of 274 ejaculates were collected and processed. The volume of the ejaculate and the initial motility was estimated immediately after each collection. The concentration of spermatozoa was determined using haemocytometer. The live and dead percentage was estimated as per Blom (1950). After assessing the ejaculates for quantity and quality, it was diluted in tris-egg yolk extender containing antibiotics. The diluted semen was stored in a refrigerator and at periodic intervals semen samples were examined up to 72h for the motility under the microscope using a biotherm.

The fertility trials were carried out in the institute farm animals using chilled semen. The pregnancy was confirmed by rectal palpation after 60 to 90 days of insemination.

FREEZING TECHNIQUE: (Chinnaiya and Ganguli 1980)

a) The freshly collected semen was evaluated for quantity and quality

TABLE 1: Semen Characteristics of Crossbred Bulls

Bull No.	No. of ejaculates	Average volume (ml)	sperm concentration (millions per ml.)	Initial motility (percent)	Live sperm (percent)
400	94	5.29±1.33	915±265.29	70.12±10.12	76.94±5.09
409	84	6.56±1.64	1320±632.94	80.54±6.80	76.58±5.60
415	96	6.19±1.19	1210±612.08	80.00±6.94	81.6±7.03
OVERALL MEAN: (n=274)		6.0	1148.0	77.08	78.37

TABLE 2: Average Motility (%) of Crossbred Bull Semen on Storage at 5-7°C.

Bull No.	No. of observations(n)	0 hour	24 hours	48 hours	72 hours
400	94	70.12±6.09	63.49±12.66	53.68±16.83	46± 8.43
409	84	80.54±6.80	76.06±9.93	66.49±19.23	59.09± 7.65
415	96	80.0 ±7.03	65.52±23.72	64.34±12.99	60.83±11.00
OVERALL MEAN: (n=274)		77.08	68.00	61.20	55.30

and samples were selected for further processing.

- b) The semen was diluted in tris-egg yolk extender containing 7% glycerol.
- c) Gradually the temperature of the diluted semen was lowered to 5°C in a period of 45 m by adding ice pieces.
- d) The glycerolated semen was kept at 5°C for a period of 4h for equilibration.
- e) The glycerolated and equilibrated semen was packed in pre cooled 0.5 ml plastic straws.
- f) The straws were placed horizontally on the freezing rack about four centimeter above the level of liquid Nitrogen in a biological freezer for 10 m.
- g) The straws were transferred into liquid Nitrogen container and stored.
- h) Straws were picked up at random, thawing was done in water at 38°C for 2 to 4 m and examined for motility under phase contrast microscope using biotherm.

Results and Discussion

The mean values of various physical characteristics of semen are presented in table 1. The mean volume per ejaculate was found to be 6.0 ml. The value obtained in the present study is higher than the values reported (4.33 ml) by Narasimha Rao and Ramamohan Rao (1978), for Brown Swiss×Ongole and Holstein Friesian × Ongole bulls. The difference in the volume of semen may be due to variations in age and body weight of the bulls in their respective studies. The average initial sperm motility was 77%. The values in the present study are comparable to the values reported by Roy *et al.*, (1975) in crossbred bulls. The average concentration of spermatozoa recorded in the present study was 1148 millions per ml of semen which is in agreement with the results of Biswas *et al.*, (1978). The average live sperm count was 78.37%. The higher volume per ejaculate and the low sperm counts obtained from the present studies might be due to the genotypic differences of

TABLE 3. Agerage Conception rate using the Crossbred Bulls Semen

Bull No.	No. of inseminations	No. of pregnancies	Conception (%)
400	130	43	33.1
409	162	59	36.4
415	106	44	41.5
OVERALL MEAN: n=398		n=146	37.0

the crossbred bulls used.

The effect of storage in diluted semen at refrigeration temperature is presented in table 2. It could be observed that average decline in the motility was gradual except in bull No 400 which showed rapid deterioration. In this bull, in particular the keeping quality and discard rate due to low motility were higher than other bulls. Saxena and

Tripathi (1981) observed similar trends in the studies on seasonal variation in semen characteristics and preservability in crossbred bulls.

The percentage of conception using chilled semen of crossbred bulls is presented in table 3. The conception rate of bull No 400 was the lowest (33.1%) compared to the overall percentage of conception (37%) in the present

TABLE 4. Details of Freezing of Crossbred Bull Semen

Bull No.	No. of trials	Motility in percent			Remarks
		Before freezing	After freezing		
			Immediately	One month of storage	
400	1	70	30	30	preserved
	2	50	5	—	discarded
	3	50	Not fit for freezing		— do —
	4	45	— do —	—	— do —
	5	65	— do —	—	— do —
	6	60	— do —	—	— do —
	7	60	— do —	—	— do —
409	1	65	35	30	discarded
	2	60	50	30	— do —
	3	80	40	40	preserved
	4	90	50	40	— do —
	5	70	50	40	— do —
	6	75	40	40	— do —
	7	70	50	40	— do —
415	1	70	40	40	preserved
	2	50	40	25	discarded
	3	80	50	30	— do —
	4	80	40	40	preserved
	5	70	40	30	discarded
	6	65	40	40	preserved

OVERALL MEAN PERCENTAGE OF DISCORD=45 (n=20)

study. The poor conception rate is comparable to the values (40.8%) reported by Rao and Rao (1979) in Brown Swiss \times Ongole and Holstein Friesian \times Ongole bulls. Kaikini *et al.* (1981) reported similar findings (36.28%) in Holstein Friesian crossbred bulls.

The data on freezability of crossbred bull semen are presented in table 4. It was observed that the freezability was poor and the discard rate was more than 45%. A high rate of discard with poor freezability was reported by Mathew

(1976). Further study, with greater number of freezing trials, is suggested to draw definite conclusions on the crossbred bulls semen.

Acknowledgement

The authors wish to thank Dr. T. G. Venkataraman and Sri. L. Krishna Murthy for their able assistance in laboratory analysis. Further the authors gratefully acknowledge the facilities provided by Dr. P. G. Nair, Head, Southern Regional Station, National Dairy Research Institute, Bangalore.

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Effect Of Synthetic Oxytocin On Some Of The Seminal Attributes In Cow And Buffalo Bulls

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ABSTRACT

An attempt was made to study the effect of administration of synthetic oxytocin upon some of the seminal characteristics in ox bulls and buffalo bulls. Analyses revealed that injection of oxytocin prior to ejaculation reduced the reaction time, pH and increased the volume, mass motility, sperm concentration and percentage of live spermatozoa in the semen. The results were discussed.

* * *

Oxytocin is a posterior pituitary peptide hormone and considered to be responsible for the stimulation of ejaculation. Composition of the ejaculate and willingness to copulate in male rabbits has been reported to be increased upon intravenous injection of purified posterior pituitary extract (Kihlstrom and Melin, 1963; Melin and Kihlstrom, 1963). Oxytocin influences the seminal picture by inducing contraction of testes and epididymis (Bereznev, 1963). In the present paper an attempt has been made to study the effect of oxytocin on some of the seminal attributes in ox bulls and in he-buffalo bulls.

Materials and Methods

Five Holstein-Friesian and three buffalo bulls maintained at dairy farm were considered for the study. Semen samples

were collected from each bull biweekly without giving any treatment and following characteristics of semen viz., reaction time, volume, motility, sperm concentration, live percentage and pH were studied as per Tomar (1970). Similarly, ejaculations were taken from each bulls within five minutes after intramuscular injection of 2 ml Unitocin (Sparterine sulphate 150 mg per ml-Unichem Laboratories, Bombay), a synthetic oxytocin preparation and all above parameters were studied to note the effect of the drug on seminal picture of these bulls. The data were analysed (Snedecor and Cochran, 1967).

Results and Discussion

The analysis revealed that after treatment there was reduction in reaction time in both the bulls and buffalo bulls. The average reaction time before treatment was 43.43 seconds and 55.90 seconds in ox bull and buffalo bulls respectively, while after the treatment, the average reaction time reduced to 26.60 seconds and 43.66 seconds respectively. In ox bull No. 2 and 3 the difference in reaction time was found statistically significant, however, in the other bulls this difference was not statistically significant which may be due to individual differences among the bulls.

TABLE 1: Mean values of various seminal characteristics before and after the treatment in Holstein-Friesian and buffalo bulls

	Total No. of obser- vations	Reaction time (Seconds)	Volume (ml)	Mass motility	Sperm concen- tration (millions/ml)	Per centage of Live spermatozoa	pH
<i>Holstein-Friesian bulls</i>							
Before treatment	50	43.43	4.01	+3.2	795	78.16	6.75
After treatment	50	26.66	4.41	+3.66	947	81.32	6.72
<i>Buffalo bulls</i>							
Before treatment	30	55.90	1.75	+2.96	724	80.43	6.82
After treatment	30	43.60	1.92	+3.43	789	81.73	6.72

TABLE 2: t-value of different seminal characteristics in response to oxytocin administration

Bull No. Species	Reaction time	Volume	Mass motility	Sperm concentration	percentage of live spermatozoa	pH
<i>Holstein-Friesian</i>						
Bull No. 1	1.921	1.10	3.68**	0.94	0.42	4.53**
Bull No. 2	3.100*	1.40	6.01**	1.47	0.07	0.00
Bull No. 3	0.000	0.09	1.50	2.77*	2.64*	8.50**
Bull No. 4	1.760	0.64	4.58*	3.40**	1.62	0.75
Bull No. 5	3.640*	0.65	1.40	0.54	0.98	0.00
<i>Buffalo bulls</i>						
Bull No. 1	0.029	0.194	2.45*	0.001	0.000	1.66
Bull No. 2	2.200	0.260	6.015**	1.200	0.094	4.03**
Bull No. 3	0.380	1.230	1.500	0.4600	2.130	2.27

* The means are statistically different at 5% level of probability

** The means are statistically different at 1% level of probability

The average volume of semen obtained prior to the treatment in ox and buffalo bulls was 4.01 ml and 1.75 ml while after the treatment the volume increased to 4.41 ml and 1.92 ml respectively. There was increase in the seminal volume after the injection of oxytocin almost in all animals but the difference has not been found statistically significant. Increase in volume of semen after the injection of oxytocin in bulls and bucks has been reported (Bereznev, 1963); Fjellstrom *et al.*, 1968).

The average mass motility was as +3.2 and +2.96 before the treatment and +3.66 and +3.43 after the treatment in ox bulls and buffalo bulls respectively. The difference was found to be statistically significant in most of the bulls.

The average sperm concentration was 795 and 724 millions per ml before treatment in ox bulls and buffalo bulls respectively. However, after the treatment sperm concentration increased to 947 and 789 millions per ml. The difference in sperm concentration before and

after the treatments was found statistically significant in ox bull No. 3 and in other bulls though, there was increase in sperm concentration after the administration of oxytocin but the difference was not found statistically significant which could be due to difference in the physiological make up of animals. Increase in sperm concentration after injection of oxytocin has been reported in ram (Ewy and Bielanski, 1962) and in rabbit buck (Fjellstrom *et al.*, 1968). Roberts (1971) has also reported an increase in semen volume and sperm concentration in some bulls treated with oxytocin prior to collection of semen.

The average percentage of live spermatozoa in ox bull and buffalo bulls prior to treatment was 78.16 and 80.43 while after the treatment the live spermatozoa

percentage increased to 81.32 and 81.73 respectively. From the results it is apparent that there is increase in live spermatozoa percentage which may be attributed to the oxytocin administration. However, in only one ox bull this difference was found statistically significant.

The average pH of semen prior to treatment was 6.75 and 6.82 in ox bulls and buffalo bulls respectively while after the treatment the average pH was reduced to 6.72 in both the groups of bulls. The difference was found statistically significant in two ox bulls and in one buffalo bull.

Acknowledgement

Authors are thankful to Dr. C. M. Singh, Director, Indian Veterinary Research Institute, Izatnagar —U.P. for the facilities.

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Studies On Bacterial Flora Of Bovine Semen And Their Antibiotic Spectra

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ABSTRACT

A total of 113 neat semen samples of breeding bulls used for artificial insemination (A.I.) were investigated bacteriologically. One hundred four samples recovered isolation of either one, two or three types of organisms. The rest nine samples were found sterile for bacterial organisms. From the positive samples totally 124 bacterial isolates were obtained which included *Staph. aureus*, *Staph. epidermidis*, *Micrococcus* spp., *E. coli*, *Pr. vulgaris*, *Enterobacter cloacae* and other gram negative organisms in minor proportion.

The results of drug sensitivity test revealed that as compared to gram negative organisms, gram positive organisms were more susceptible to the drugs tested. Among the individual organisms, *Micrococcus* spp. showed least resistance while *Ps. aeruginosa* showed highest resistance. Among the drugs, gentamycin and chloramphenicol were found to be most effective, while penicillin, tetracycline and furazolidone were found less effective. Streptomycin showed satisfactory results, hence it can be continued to be added in semen dilutors.

* * *

The quality of semen is the most important single decisive factor in success or failure of our cattle development

programme through A.I. Among the several factors involved in the quality of semen, microbial contamination is the most important factor. If microbial load exceeds the permissible limit i.e. 500 bacteria per ml of frozen semen (Willis, 1978), it deteriorates semen quality. Further, contaminated semen acts as source of infection to female animals which in turn leads to infertility or sterility in them. In view of limited information available on bacterial flora of semen and their drug resistance pattern in India, the present study was undertaken to provide guidelines for proper treatment of semen dilutors so as to control microbial load of semen and in turn, to control infectious infertility due to infected or contaminated semen.

Materials and Methods

The breeding bulls stationed at three A.I. Centres belonging to Department of Animal Husbandry, Gujarat State were used in present study. Totally 113 semen samples from 45 bulls were collected during one year period. Approximately 1.5 ml. of semen was collected from each bull. Primary isolation of organisms was done on blood agar. On the basis of cultural characters and morphology of stained smears, the gram positive organisms were identified as per Bergey's manual of determinative bacteriology

TABLE 1: Antibiotic sensitivity pattern of different organisms isolated from bovine semen

Name of organism	No. of isolates tested	Number of isolates sensitive to							
		Am	C	Fx	Gm	N	P	S	T
<i>Staph. aureus</i>	4	4	4	1	4	2	1	2	2
<i>Staph. epidermidis</i>	15	15	15	3	15	14	3	11	11
<i>Micrococcus</i> Spp.	6	5	5	4	6	4	3	5	5
<i>Streptococcus</i> Spp.	10	10	10	6	10	6	3	7	1
<i>Corynebacterium</i> Spp.	3	3	3	0	3	2	1	1	2
<i>Bacillus</i> Spp.	23	13	21	19	23	18	7	21	13
	61	50	58	33	61	46	18	47	34
<i>Ps. aeruginosa</i>	18	1	5	0	18	6	0	8	0
Gram negative bacilli (Except <i>Ps. aeruginosa</i>)	45	15	33	21	37	29	5	35	18
	63	16	38	21	55	35	5	43	18

Am = Ampicillin
C = Chloramphenicol
Fx = Furazolidone
Gm = Gentamycin

N = Neomycin
P = Penicillin
S = Streptomycin
T = Tetracycline

(Buchanan and Gibbons, 1974 and Cowan and Steel, 1970), while gram negative organisms were identified using strips as well as criteria tables especially devised by the Department of Bacteriology and Hygiene at Royal Veterinary College, Copenhagen.

The bacterial isolates were tested *in vitro* against ampicillin (10 mcg), chloramphenicol (30 mcg), furazolidone (300 mcg), gentamycin (10 mcg), neomycin (30 mcg), penicillin (10 units), streptomycin (10 mcg) and tetracycline (30 mcg) using antibiotic discs supplied by Pasteur Biological Laboratories as per the method recommended by Buer *et al.*, (1966). The results were interpreted as sensitive, moderately sensitive and resistant on the basis of zone size inter chart furnished by laboratory.

Results and Discussion

A total of 113 semen samples were examined, of which 86 samples yielded single type of organisms, 16 samples revealed two types of organisms and two

samples resulted in isolation of three types of organisms. The rest nine samples were found bacteriologically negative. The isolates included *Staph. aureus* (4), *Staph. epidermidis* (15), *Micrococcus* spp. (6), *Streptococcus* spp. (10), *Corynebacterium* spp. (3), *Bacillus* spp. (23), *Ps. aeruginosa* (18), *E. coli* (6), *Pr. vulgaris* (5), *Enterobacter cloacae* (5) and variety of other gram negative organisms in minor proportion. These organisms are distributed in nature and have been reported to be associated with a wide variety of female reproductive disorders in bovine (Roberts, 1971; Barth *et al.*, 1976; Panangala *et al.*, 1978; Sharma *et al.*, 1978; Weisser, 1981; Shah and Dholakia, 1982). Therefore, their presence in semen should be viewed with caution and proper hygienic condition and care should be exercised during the collection and preservation of semen.

The isolates have shown wide variation in their sensitivity to drugs ranging from fully sensitive to completely resistant (Table 1). As compared to gram negative

organisms, gram positive organisms were found more susceptible to all the drugs tested. Among the individual organisms, *Micrococcus* spp. showed least resistance, while *Ps. aeruginosa* showed highest resistance to the drugs tested except gentamycin to which all the 18 cultures of *Ps. aeruginosa* were found to be sensitive. Korudzhiiski (1980) has also reported gentamycin to be most effective on *Ps. aeruginosa*, while all his 66 cultures of *Ps. aeruginosa* were resistant to penicillin, tetracycline, neomycin, chloramphenicol, streptomycin and furazolidone.

The results of drug-wise resistance pattern (Table 2) reveal that gentamycin was found to be most effective (93.55%) followed by chloramphenicol (77.42%), streptomycin and neomycin (each 65.33%), ampicillin (53.23%), furazolidone (43.53%), tetracycline (41.93%) and penicillin (18.55%). These findings point out that penicillin, tetracycline and furazolidone were less effective than the other antibiotics, as at present they are widely used in Veterinary practice. Combination of streptomycin and penicillin is routinely used to treat the semen

dilutor, however, adequate sensitivity was observed to streptomycin.

The purpose of the present work was to provide guideline so as to select proper drugs for treating semen dilutors. Accordingly based on antibiogram of the isolates obtained in the present study, treatment of semen dilutors can be successfully carried out by any antibiotic, provided that the drug should not have any harmful effect on spermatozoa. Isolates have shown adequate sensitivity to streptomycin, hence it can be continued to be added in dilutors to control microbial load of semen.

Acknowledgement

The authors are thankful to Dr. T. N. Vaishnav, Ex. Director of Animal Husbandry, Gujarat State for giving permission for collection of materials and interest shown in the work. The facilities and encouragement extended by Dr. M. R. Patel, Dean, Faculty of Veterinary Science and Animal Husbandry, GAU are gratefully acknowledged. Technical help given by laboratory technicians is also duly acknowledged.

TABLE 2: Effectiveness of the antibiotics on isolates

Sr. No.	Name of the antibiotic	Sensitive		Moderately sensitive		Resistant	
		No. of isolates	percentage	No. of isolates	percentage	No. of isolates	percentage
1.	Gentamycin (Gm)	116	93.55	0	0.00	8	6.45
2.	Chloramphenicol (C)	96	77.42	0	0.00	28	22.58
3.	Streptomycin (S)	80	64.52	1	0.81	43	34.68
4.	Ampicillin (Am)	66	53.23	0	0.00	58	46.77
5.	Furazolidone (Fx)	49	39.52	5	4.03	70	56.45
6.	Tetracycline (T)	50	40.32	2	1.61	72	58.06
7.	Neomycin (N)	41	33.06	40	32.26	43	34.68
8.	Penicillin (P)	16	12.90	7	5.65	101	81.45

No. of isolates tested = 124

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SHORT COMMUNICATIONS

IJAR 6: 2: 82-84, 1985

Total Sperm Out Put In Successive Ejaculates Till Exhaustion And Its Relationship With Testicular Measurements In Cross-Bred Bulls

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Kupper Schmied *et al.* (1975), Almquist *et al.* (1976), Salam Daudu and Shoyinka (1983), and Jakubiec (1984) have shown positive correlations of scrotal circumference and testicular size with sperm production in Simmental, Holstein Friesian, Nigerian Bunaji and Sokoto Gudali and Polish black and white cattle.

It could be of interest to establish whether such a correlation also existed in cross-bred cattle (*Bos taurus* × *Bos indicus*).

Nine adult cross-bred bulls of 75% exotic Holstein Friesian inheritance were involved in the study. Measurements of scrotal circumference, scrotal volume and testicular height were taken

as per Bhosrekar *et al.*, (1980) for each bull before every exhaustion by taking successive ejaculates in artificial vagina. The criterion of exhaustion was that the bull's collection was taken till he refuses to mount further even on rest for 15 to 20 minutes and the last ejaculate collected shows less than 50×10^6 sperms/ml. Average of 5 ejaculates (ranging from 3 to 10) were collected before the bulls got totally exhausted. Three such exhaustion tests were carried out for each bull.

The analysis of covariance was (Tables 3a, b, c) done to test the effect of bulls, replicates and scrotal measurement on ejaculate volume, sperm concentration

TABLE 1. Means of seminal attributes from 3 exhaustion tests

Sr. No.	Bull No.	Semen volume ml.			Sperm concentration/ml 10^6			Total sperm out put 10^6		
		1st Ejaculate	1st+2nd Ejaculates	All Ejaculates	1st Ejaculate	1st+2nd Ejaculates	All Ejaculates	1st Ejaculate	1st+2nd Ejaculates	All Ejaculates
1.	2	3.33	6.77	12.93	3375	2543	1868	12225	18275	24196
2.	3	7.20	12.60	29.60	2400	1903	1313	17370	24910	36747
3.	4	6.33	12.70	31.76	2850	1901	1201	18580	24870	36230
4.	10	6.73	12.40	32.10	2308	1790	977	15918	22272	30878
5.	11	4.47	7.80	12.40	1648	1314	1175	7290	9918	14608
6.	12	4.73	7.73	23.57	2645	2002	1330	12274	15618	27432
7.	13	9.13	17.00	44.33	1907	1316	906	18032	22785	39414
8.	026	4.27	6.67	19.80	1742	1518	1126	7500	10181	22370
9.	244	7.47	12.53	20.73	1767	1265	946	13120	15657	19224

TABLE 2. Mean values of scrotal measurements

Sr.No.	Bull No.	Scrotal volume ml.	Scrotal circumference cm.	Testicular height cm.
1.	2	1281.67	34.00	13.75
2.	3	1217.33	34.53	14.92
3.	4	1291.67	33.00	16.17
4.	10	1375.00	36.93	14.42
5.	11	1550.00	39.17	15.25
6.	12	1356.67	36.20	16.17
7.	13	1433.33	34.87	14.92
8.	26	1076.67	37.50	15.67
9.	244	1566.67	40.53	20.25

TABLE-3 (a). Analysis of covariance: covariate scrotal volume

Source of variance	D.F.	Ejaculate volume Mean Squares	Sperm concentration Mean Squares	Total sperm number Mean Squares
Between bulls	8	315.84**	256116.01	219591790.00**
Between replicate	2	70.32	451935.60	229007332.00**
Regression	1	1.11	38063.56	375389.42
Error	15	47.20	135422.97	29404017.00

TABLE 3(b). Analysis of covariance: covariate scrotal circumference

Source of variance	D.F.	Ejaculate volume Mean Squares	Sperm concentration Mean Squares	Total sperm numbers Mean Squares
Between bulls	8	315.84**	256116.01	219591790.00**
Between replicate	2	70.32	451935.60	229007332.00**
Regression	1	19.48	54561.63	2063066.50
Error	15	45.98	134323.10	29291505.00

TABLE 3(c). Analysis of covariance: covariate testicular height

Source of variance	D.F.	Ejaculate Volume Mean Squares	Sperm concentration Mean Squares	Total sperm numbers Mean Squares
Between bulls	8	315.84**	256116.01	219591790.00**
Between replicate	2	70.32	451935.60	229007332.00**
Regression	1	21.68	813.70	465115.31
Error	15	45.83	137906.29	29398035.00

per ml. and total sperm out put. The results showed highly significant differences between bulls for all the parameters, which was also expected because of the individual variation amongst the bulls. There was no significant variation for ejaculate volume and sperm concentration per ml. However, there was highly significant difference for total sperm out put between replicates. This could be because of variable number of ejaculates collected before the bulls got exhausted in each replication. The critical differences showed 2nd replicate having significantly higher sperm output.

The regressions calculated for total

semen volume, sperm concentration/ml and total sperm out put over scrotal volume, scrotal circumference and testicular height showed no statistical significance.

From this study it could be concluded that in cross-bred bulls scrotal measurements could not be taken as criteria for selecting bulls for semen production.

Acknowledgement

The authors are indebted to Dr. Manibhai Desai, the Director and Dr. D. S. Gorhe, the Chief Executive for providing all facilities and permission to publish the data.

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Cryopreservation Of Buffalo Semen In Lactose-Fructose Extender With Different Levels Of Glycerol

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ABSTRACT

Cryopreservation of Surti buffalo semen in lactose-fructose extender with different levels of glycerol is reported. Lactose-fructose at 312 mM/lit in a semen extender without a buffer showed no significant variation in initial and prefreeze motilities between 0, 1.5, 3.0 and 6.0 per cent glycerol levels.

The initial and prefreeze motility in extenders containing varying levels of glycerol were lower than the control (Tris extender) and it was significant ($p < 0.05$) in initial motility. The post thaw motility increased significantly ($p < 0.05$) as the level of glycerol in the experimental extender increased from 0 to 3 per cent and its increase between 3 to 6 per cent glycerol level was not significant, but it was lower than the tris extender.

* * *

Glycerol has been extensively used as cryopreservative for bovine spermatozoa frozen in pellets, ampules and straws (Martin *et al.*, 1965; Nagase *et al.*, 1964). In *Bos taurus* bulls there is ample literature on the effect of various levels of glycerol in frozen semen extenders, whereas in buffaloes this information is scanty. However, Rathore (1965) observed very low survival rate of Murrah buffalo spermatozoa in instantaneous freezing to -79°C and 7 per cent glycerol gave better

results than 14 per cent glycerol in yolk citrate diluent. In 0.5 ml French straws, the buffalo spermatozoal motility was better with 6 per cent glycerol when dilution rate was 1:10 and with 8 per cent glycerol when the dilution rate was 1:20 in egg yolk citrate, citric acid whey and tris diluents (Chinnaiya and Ganguli, 1980). In freezing of white cattle semen glycerol is indispensable and sugars are some times complimentary and useful for cryopreservation of spermatozoa (Salisbury *et al.*, 1978). In the present study an attempt has been made to know the effect of different levels of glycerol in an egg yolk-lactose-fructose extender without a buffer for freezing of buffalo semen in straws.

Materials and Methods

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

The composition of the experimental extender was as follows:

Stock solution:

11 per cent lactose solution 56.25 ml
229 mM

6 per cent fructose solution 18.75 ml
83 mM

Total 312 mM

TABLE 1: The mean percentage values of initial motility, prefreeze motility and post thaw motility in lactose-fructose-glycerol extenders. (% in arcsine values).

Glycerol per cent	Initial motility	pre-freeze motility	Post-thaw motility
0	56.24 \pm 0.90 ^a	53.22 \pm 1.17 ^a	21.82 \pm 1.31 ^a
1.5	55.84 \pm 1.01 ^a	53.03 \pm 1.22 ^a	28.43 \pm 1.22 ^b
3	55.63 \pm 0.97 ^a	52.84 \pm 1.32 ^a	33.72 \pm 1.85 ^c
6	55.42 \pm 0.92 ^a	52.62 \pm 1.19 ^a	35.03 \pm 1.58 ^c
Control tris extender	59.87 \pm 0.81 ^b	56.28 \pm 1.10 ^a	36.25 \pm 1.71 ^c

Means with common superscript do not vary significantly at $\leq p$ 0.05.

Extender composition:

Egg yolk 20 per cent

Glycerol 0, 1.5, 3.0 or 6.0 per cent

Stock solution to 100 ml.

Tris-citric acid fructose egg yolk glycerol extender (Vasanth, 1978) which is routinely used in the buffalo breeding project was taken as control extender. Split semen samples were diluted in different experimental 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 polyvinyl alcohol (PVA) powder. Identification of straws with different extenders was done utilizing different combinations of color of straws and PVA powder. After four hours of equilibration period, the straws were frozen over liquid nitrogen vapour for 10 minutes in Minnesota Valley Engineering Biological Freezer and then plunged into liquid nitrogen. The straws were collected from the freezer and stored in frozen semen container. The initial motility (immediately after extension of semen) pre-freeze motility (after 4 hours of equilibration) 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 trisodium citrate solution (3 per cent) adjusted to pH 7 with citric acid to semen samples as described by Flukes *et al.* (1977). The percentage values of motility was 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 initial and prefreeze motility (Table 1) in lactose-fructose extender did not vary significantly without and with different levels of glycerol. Further the initial and prefreeze motility were lower than the control (tris extender) but it was only significant ($p \leq 0.05$) in the initial motility (Table 1). The post thaw motility increased significantly ($p \leq 0.05$) from 0 to 3 per cent glycerol level in lactose-fructose extender. Further 3 and 6 per cent glycerol level though gave lesser post thaw motility than control (Table 1) it was not significant.

Discussion

In cryopreservation of bull semen in straws, fructose was not beneficial in absence of glycerol, whereas lactose was beneficial in absence or presence of glycerol at low concentration and no combination of glycerol and lactose was

superior to glycerol alone (Unal *et al.*, 1978).

In buffaloes there is no information on the effects of varying levels of glycerol in semen extenders containing lactose-fructose sugars without buffer. However, egg yolk lactose extender with 4.7 per cent glycerol gave 43.3 per cent post thaw motility for buffalo semen frozen in straws, but tris extender was better than egg yolk lactose glycerol extender (Singh, 1977).

In Nili-Ravi buffaloes a constant 5 per cent glycerol level in lactose or fructose alone or in different combinations but without a buffer gave significant variation in post thaw motility (Ahmed and

Chaudhary, 1980). The lesser post thaw motility in the lactose-fructose extender with different levels of glycerol than the control (tris extender) in the present study (Table 1) could probably be due to absence of a buffer. If the buffer salt concentration is lowered below the minimum amount necessary to maintain the pH level during a storage period the survival of spermatozoa will be dramatically affected (Kampschmid *et al.*, 1953). It could be concluded that lactose-fructose sugars at 312 mM in an extender containing different levels of glycerol do not provide adequate cryoprotection in absence of a buffer.

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Studies On Certain Biochemical Constituents Of Epididymis Of Male Goats

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The ability of the epididymis to produce and secrete the various organic compounds fluctuates characteristically between different regions of the epididymal duct. This is shown by chemical analysis of luminal contents taken from different segments of the organ, and by histochemical and radiographic observations made on the epididymal epithelium directly. Biochemical analysis have been made on the epididymal fluid of the bull, boar, stallion, guinea pig, rat and ram. The characteristic feature of the epididymal plasma is that its combined ionic content is considerably weaker than that of testicular or blood plasma.

Crabo and Gustafsson (1964) and Crabo (1965) observed sodium, potassium and total protein concentration in six different regions of the epididymis of bulls and boars respectively. Tuck *et al.* (1969) compared the rete-testes fluid from seminiferous tubules. Einarsson (1971) compared the sodium, potassium, magnesium, and total protein concentration in cauda epididymal fluid with the different fractions of semen in the boars. Turner *et al.* (1977) obtained fluid by using micropuncture technique of rat

epididymis. Mann and Mann (1981) reported gradual increase in magnesium concentration from testicular plasma towards cauda epididymal fluid in the bovine.

Materials and Methods

The epididymis along with testicles of 18 mature local bucks were collected from slaughter house, Mhow. The fluid from ductuli efferentes (DE), caput, corpus and cauda epididymis was collected with the help of pasteur pipette and than pooled. One pooled sample represents material from six animals. The sodium and potassium concentration was determined with the help of an EEL flame photometer as described by Oser (1965). The magnesium concentration was determined by the method of Spare (1962). The total protein was estimated by Biuret method as described by Oser (1965). The standard statistical methods as described by Snedecor and Cochran (1967) were used for analysis of data.

Results

The mean and standard error of sodium, potassium, magnesium and total protein is presented in table.

TABLE: Mean and standard error of different biochemical constituents

Constituents	Ductuli efferentes (DE)	Caput	Corpus	Cauda
Sodium (mEq/lit.)	93.00±1.527	79.333±2.333	77.00±1.527	41.333±0.666
Potassium "	19.00±2.081	30.00±2.886	48.666±1.333	28.333±1.666
Magnesium (mg/100 ml)	1.204±0.042	3.333±0.291	1.783±0.015	1.516±0.115
Total protein (gm/100 ml)	1.166±0.165	2.333±0.165	2.466±0.033	2.933±0.132

The sodium concentration decreased significantly ($P < 0.05$) towards the cauda epididymis and minimum was noted in cauda epididymis. The concentration of potassium was maximum in corpus. The highly significant difference ($P < 0.01$) was noted in different segments regarding potassium concentration. The ratio of sodium: potassium was highest in DE then decreased significantly ($P < 0.05$). The highly significant difference ($P < 0.01$) was noted in different segments regarding magnesium concentration and maximum was in caput epididymis. The total protein concentration gradually increased significantly ($P < 0.05$) from DE towards cauda epididymis.

Discussion

The concentration of sodium in the present study is in accordance with the reports of Crabo (1965) and Salisbury and Cragle (1956) in bulls. Einarsson (1971) also reported similar findings in cauda epididymal fluid of boars. As regards potassium concentration the values are in accordance with the reports of Crabo (1965) in bulls and boars and by Scott *et al.* (1963) for ram epididymis. However, the present value differ with the reports of Salisbury and Cragle (1956) for bulls. The maxi-

mum ratio of sodium: Potassium was noted in DE which agrees with the findings of Crabo (1965) in bulls. Lowest ratio was found in cauda as also reported by Scott *et al.* (1963) in ram and Einarsson (1971) in boar epididymal fluid. In the bulls the sperm spend the greater part of their maturity in an environment with a relative low sodium: potassium ratio (Crabo, 1965).

The magnesium concentration in the present study was in close agreement with the values reported by Scott *et al.* (1963) in ram and Einarsson (1971) in the cauda epididymis of boar. It is difficult to grade the significance of the electrolyte content for the metabolism and motility of spermatozoa but striking feature is that there is high sodium and magnesium concentration and low potassium concentration in seminal plasma in relation to the caudal plasma (Einarsson, 1971).

The total protein concentration in the present investigation are in agreement with the findings of Crabo (1965) and Mann and Mann (1981) in bulls and Einarsson (1971) for boars. However, Scott *et al.* (1963) reported lower values as compared to present findings.

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Characters Of Semen And Lactic Acid Profile At Different Hours Of Preservation In Jamnapari And Barbari Bucks

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ABSTRACT

The present study was conducted to record the breed differences for various seminal characteristic of buck semen. Significant ($P < 0.01$) effect of breed was observed on motility percentage, and sperm concentration of neat semen. The effect of breed was also found to be significant ($P < 0.01$) on motility percentage and percentage of live spermatozoa at different hours of preservation. Lactic acid content had negative significant correlation with percentage of motile and live spermatozoa in both the breeds (Jamnapari and Barbari).

* * *

There is high correlation between quality of semen and fertility and therefore it is necessary to evaluate the characters of semen before it is used for breeding. In recent years interest in goat breeding has increased for various diverse reasons. Main reasons ostensibly are meat and milk production. This has warranted more informations on the breeding worth of the bucks and their quality of semen. A few reports on seminal characters of Jamnapari and Barbari bucks are available (Patel, 1967; Sahni and Roy, 1969; Mittal and Pandey, 1972 and Singh *et al.* 1982)., Therefore, the present study was undertaken to record breed differences in regard to seminal characters of Jamnapari and Barbari bucks. Record-

ings have also been made on the relationship of lactic acid content with other seminal attributes.

Materials and Methods

Altogether 68 semen samples collected twice weekly from 5 Jamnapari and 3 Barbari bucks by means of an artificial vagina were included in this study. Of these 10 seminal ejaculates were collected from each of Jamnapari buck and 6 from each of Barbari buck. Bucks were of approximately same age and were kept under similar managerial conditions. Immediately after collection semen samples were assessed for volume, initial motility, live sperm percentage and sperm concentration. For lactic acid determination (Barker and Summerson, 1941) one ml of breedwise pooled semen was taken and was deproteinized immediately. Pooled semen samples were extended in egg yolk citrate (Salisbury *et al.*, 1941) in the ratio of 1:10 to study the effect of hours of preservation and breed on various seminal characteristics. Penicillin G sodium and Streptomycin were added at the rate of 1000 I.U. and 1000/ μ g per ml of diluted semen. Diluted semen samples were kept in refrigerator at $+5^{\circ}\text{C}$ and were evaluated at 24 hours intervals till 96 hours of preservation for (i) motility percentage (ii) Live sperm percentage (iii) hydrogen ion concentration (pH) and (iv) Lactic acid accumu-

TABLE 1. Mean values with S.E. for different characters of buck semen

Characters	B R E E D		F— Value
	Jamnapari	Barbari	
Volume (ml)	0.99±0.14 (50)	1.22±0.14 (18)	2.18
Motility Percent	66.60±3.69 (50)	87.78±2.50 (18)	10.98**
Live sperm per cent	75.30±3.33 (50)	78.33±1.76 (18)	0.11
Sperm concentration (Millions /ml)	2876.60±79.60 (50)	1976.67±71.96 (18)	41.34**
Lactic acid content (mg/100 ml)	94.02±8.29 (10)	81.04±6.27 (6)	1.03

Figures in parenthesis are number of observations

**, $P < 0.01$.

lation. Statistical analyses of the data were done according to Snedecor and Cochran (1968).

Results and Discussion

Mean values of different attributes of undiluted semen have been presented in table 1.

Ejaculate volume: The mean ejaculate volume (0.99 ± 0.14 ml) and (1.22 ± 0.14 ml) obtained from Jamnapari and Barbari bucks respectively (Table 1) are comparable with the findings of Patel (1967), Koh (1975) and Singh *et al.*, (1982). The values for Barbari bucks were slightly higher than the values reported by Sahni and Roy (1967, 1969) and Tewari *et al.*, (1968). Whereas the values for Jamnapari bucks were lower than those reported by Tewari *et al.*, (1968) but were in close accordance with those reported by Sahni and Roy (1969). Variations in the ejaculate volume might occur due to variety of factors including agroclimatic conditions, feeding, management, age, body weight and functional status of accessory sex glands of bucks (Orson and Victor, 1952; Prasad, 1969). During the present study the difference

between two breeds in regard to ejaculate volume was not significant (Table 1).

Motility percentage: Analysis of variance (Table 1) revealed that the initial motility percentage of Barbari buck semen was significantly higher ($87.78 \pm 2.50\%$) than that of Jamnapari bucks ($66.60 \pm 3.69\%$). Significant effect of breed of bucks on motility percentage at 0, 48, 72 and 96 hours of preservation was observed (Table 2). It is evident from the table that the keeping quality of Jamnapari semen in regard to motility percentage was better in comparison to semen of Barbari bucks.

Live sperm percentage: The percentage of live spermatozoa was 75.30 ± 3.33 and 78.37 ± 1.76 for Jamnapari and Barbari buck semen respectively (Table 1). The effect of breed on percentage of live spermatozoa was not significant (Table 1). The values for live sperm percentage obtained in this study are slightly lower than the values reported by Patel (1967) and Tewari *et al.*, (1968). A significant breed difference was observed for percentage of live spermatozoa of diluted semen at 0, 48, 72 and 96 hours

TABLE 2. Average values of various seminal characters at different hours of preservation

Breed/Character	Hours of preservation				
	0	24	48	72	96
Motility (%):					
Jamnapari	81.60±1.54	60.10 ± 2.85	51.4±2.24	38.20±2.60	18.70±3.05
Barbari	72.5±1.98	61.50±1.18	42.67±1.36	26.83±1.89	5.67±1.98
t-values	3.62**	0.36	2.93*	5.90**	3.03**
Live sperm (%):					
Jamnapari	89.50±1.39	70.80±2.24	59.45±1.92	47.40±2.33	25.25±3.29
Barbari	85.00±1.24	69.30±0.76	51.17±1.17	36.33±1.20	15.33±1.45
t-values	2.19*	0.50	3.08**	3.45**	2.21*
Hydrogen ion concentration (pH):					
Jamnapari	6.68±0.04	6.43±0.04	6.22±0.07	6.01±0.09	5.74±0.09
Barbari	6.79±0.02	6.57±0.01	6.40±0.02	6.21±0.01	5.95±0.07
t-values	2.01	2.45*	1.87	1.60	1.57
Lactic acid (mg/100 ml):					
Jamnapari	25.21±7.32	50.37±10.24	63.43±9.40	74.97±9.85	103.59±11.02
Barbari	17.17±2.53	26.28±2.09	41.77±3.40	60.54±3.53	89.45±7.92
t-values	0.82	1.77	1.71	1.09	0.90

* P<0.05.

**, P<0.01.

of preservation (Table 2). It is also evident from the table that the keeping quality of Jamnapari buck semen was significantly superior than Barbari when evaluated in respect of percentage of live spermatozoa.

Sperm concentration: It is evident from table 1 that the concentration of spermatozoa in Jamnapari semen was significantly ($P<0.01$) higher (2876.60 ± 79.60 millions/ml) than that of Barbari buck semen (1976.67 ± 71.96 million/ml). Several workers including (Kurian and Raja, 1965; Tewari *et al.*, 1968; Mittal and Pandey, 1972 and Singh *et al.*, 1982) have reported significant breed and buck difference in respect of sperm concentration.

Hydrogen ion concentration (pH): The values of pH of diluted Jamnapari semen (Table 2) ranged between 6.68 ± 0.04 at 0 hour of preservation to 5.74 ± 0.09 at 96 hours of preservation. Similarly the pH of diluted Barbari semen ranged be-

tween 6.79 ± 0.02 at 0 hour of preservation to 5.95 ± 0.07 at 96 hours of preservation (Table 2). The difference between breeds was not significant at all stages of preservation except when recorded at 24 hours of preservation.

Lactic acid content: The average lactic acid content of neat semen of Jamnapari and Barbari bucks was 94.02 ± 8.29 and 81.04 ± 6.27 mg/100 ml respectively

TABLE 3. Correlation coefficients of Lactic acid content with Motility (%) and Live sperm percentage

Characters	Correlation coefficients(r)	
	Jamnapari	Barbari
Motility percentage	-0.67**	-0.94**
and Lactic acid content	(50)	(30)
Live sperm percentage	-0.63**	-0.94**
and Lactic acid content	(50)	(30)

Figures in parenthesis are number of pairs of observations.

**, $P<0.01$.

(Table 1). The difference between the two breeds was not significant. The difference between two breeds for lactic acid content of diluted semen at different hours of preservation was also not significant (Table 2). Perusal of this table revealed that the quantity of lactic acid increased with the increase in hours of preservation in the semen of both the breeds. Lactic acid content had significant ($P < 0.01$) negative correlations with motility percentage and live sperm percentage in both the breeds (Table 3).

Negative correlations indicate that the percentage of motile spermatozoa and live spermatozoa decreased with the increase in the quantity of lactic acid in the semen. Determination of lactic acid content may be considered as an important test to assess the quality of semen.

Acknowledgement

Authors wish to thank Dr. H. R. Mishra, Dean, College of Veterinary Science & A.H., Ranchi for providing necessary facilities.

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Effect Of Glycerol And DMSO On Sperm Motility In Frozen Buck Semen

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Glycerol and dimethyl sulphoxide (DMSO) were observed to penetrate sperm cell rapidly and known to protect sperm from freezing damage (Pickett and Berndtson, 1978). Several workers were of the opinion that glycerol afforded better protection to ram and bull sperm during freeezing than did DMSO (Lovelock and Bishop, 1959; Salamon, 1968; Snedeker and Gaunya, 1970; Jeyendran and Graham, 1980 and Curiel and Mendez, 1981). Perusal of available literature revealed no study on effect of DMSO on freezability of buck semen. Hence, in the present investigation an attempt was made to study the relative efficacy of glycerol and DMSO for freezing of buck semen.

Twenty ejaculates consisting of 4 from each of 5 local bucks of 2 to 4 years of age were collected twice weekly using an artificial vagina. If a buck donated less than 0.8 ml of semen, a second ejaculate was collected and pooled together and considered as a single ejaculate. Four extenders containing glycerol 6.4 per cent or DMSO 4, 6.4 or 9 per cent were prepared as follows. The tris egg yolk citric acid fructose extender containing glycerol 6.4 per cent (Hahn, 1972) was prepared in two fractions. The fraction-A did not contain glycerol and fraction-B contained glycerol 12.8 per cent (V/V). In the other 3

extenders, glycerol in fraction-B was replaced by DMSO 8, 12.8 or 18 per cent (V/V). Immediately after collection, the ejaculate was diluted with the 4 extenders by split sample technique. The dilution, cooling, freezing and thawing were made as per Deka and Rao (1984). The percentage of progressively motile sperm (PPM) was estimated at two stages viz., after 5 hours of equilibration (at 4-5°C) and after 14 hours of freezing (at -196°C) using a phase contrast microscope. The statistical analysis of the data was carried out as per Snedecor and Cochran (1968) after transforming the percentages into angles.

The mean PPM after equilibration and after freezing with glycerol and different levels of DMSO is presented in Table 1. The PPM differed significantly ($P < 0.01$) between cryoprotectants, between stages (equilibration and freezing) and due to cryoprotectant X stage interaction. Critical difference test showed that PPM after equilibration and after freezing was significantly higher ($P < 0.01$) with glycerol than with DMSO and it did not differ significantly between different levels of DMSO (Table 1). The interaction between cryoprotectant and stage was significant ($P < 0.01$) because of more drop of PPM during freezing of semen with DMSO than with glycerol. The result of this study is in agreement

TABLE 1. Percentage of progressively motile sperm (Mean \pm SE) after equilibration (E) and after freezing (F) in extender containing different cryoprotectants

Cryoprotectants	E	F
Glycerol 6.4%	79.80 ^a \pm 0.72	68.50 ^b \pm 0.78
DMSO 4%	72.80 ^b \pm 1.07	90.85 ^c \pm 2.33
DMSO 6.4%	70.20 ^b \pm 1.06	30.50 ^c \pm 2.94
DMSO 9%	67.20 ^b \pm 1.06	27.25 ^c \pm 3.13

Means bearing different superscripts differ significantly ($P < 0.01$).

* 20 observations

with those of other workers on ram and bull semen (Lovelock and Bishop, 1969; Salamon, 1968; Snedeker and Gaunya, 1970; Jeyendran and Graham, 1980 and Curiel and Mendez, 1981).

It is evident from the result of this

study that DMSO was inferior to glycerol for freezing of buck semen.

Acknowledgement

The authors are grateful to the Principal, College of Veterinary Science, Tirupati for the facilities provided.

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Effect Of High Atmospheric Temperature On Semen Quality Of Young Surti Buffalo Bulls

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ABSTRACT

High ambient temperature (above 40°C) during summer appears to have a detrimental effect on mature spermatozoa of young Surti buffalo bulls. The spermatozoa are rendered immotile due to excess heat, consequently the mass activity and initial motility of the semen produced during hot summer months is significantly lowered. The deleterious effects of heat are temporary and recovery occurs during cooler months.

* * *

The influence of testicular and pituitary hormones on the process of semen generation is intimately linked with certain climatic factors especially atmospheric pressure, light and temperature. A hot climate is believed to be the principal cause of certain forms of sub-fertility among domestic animals in tropical countries (Mann, 1964). Goswami and Narain (1962) stated that high environmental temperature above 23.6°C upsets the normal physiological functions of the buffalo. High environmental temperature adversely affects spermatogenesis in the buffalo bulls. Extra heat applied to the scrotum can affect both spermatogenesis and mature sperm (Phillips and M. Kenrie, 1934). Gunn et al, (1942) reported that hot weather caused seminal degeneration in rams, and that recovery usually occurred in cooler season.

Materials and methods

The present report deals with two young Surti buffalo bulls from whom semen was collected with artificial vagina. The semen evaluation involving following parameters was done.

1. Gross evaluation-color, consistency and volume was noted.
2. Microscopic evaluation-Mass activity of neat semen and initial motility of citrate diluted semen was noted.

Data regarding atmospheric temperature during the period of study were collected from meteorology section of this station.

The efforts were made to assess the effects of high atmospheric temperature during the summer months (June and July).

Results and Discussion

As indicated in Table 1, semen quality is adversely affected in terms of mass activity and initial motility by the rise in ambient temperature. The result shows that semen quality is unaffected when the average atmospheric temperature is not above 37-38°C as during March and April months in the present study. Atmospheric temperature rose to $41.15 \pm 0.50^\circ\text{C}$ during the month of May but it did not have a sudden effect on the quality of semen, the quality of semen started

TABLE 1. Atmospheric temperature and semen quality of two young Surti buffalo bulls during March to August period.

Month		March 1985	April 1985	May 1985	June 1985	July 1985	August 1985	
Atmospheric temperature (°C)	Maximum Range	34.0—38.1	31.5—40.5	34.6—43.9	36.5—40.5	27.0—37.0	24.0—32.0	
	(Mean)	(36.79±0.198)	(38.80±1.002)	(41.15±0.50)	(40.05±1.32)	(32.44±1.34)	(29.543±0.278)	
	Minimum Range	8.5—21.3	15.7—25.2	22.0—28.0	25.0—28.0	22.0—26.7	21.0—24.3	
	(Mean)	(13.70±0.73)	(20.6±0.52)	(25.56±1.43)	(26.689±0.61)	(24.35±0.24)	(22.432±0.79)	
Bull No. (Two bulls A and B)	A	B	A	B	A	B	A	B
No. of collections	7	—	4	1	5	7	6	9
Volume (ml) (Mean)	1.14	—	1.175	3.00	1.30	2.78	1.25	3.39
Colour	white	—	white	white	white	white	white	white
Consistency	creamy	—	creamy	milky	1)creamy 2)watery	1)milk 2)creamy	1)watery 2)milky 3)creamy	creamy
Mass activity: Range (0—5 grade)	3.0—4.0	—	—	—	0—4	2—4	0—1	0—4
(Average)	(3.71)	—	(4.0)	(3.0)	(2.2)*	(3.14)*	(0.166)**	(1.44)**
Initial motility % : Range	65-90	—	—	—	0—85	50—80	10—80	0—70
(Average)	(77.14)	—	(80)	(70)	(46)*	(59.64)*	(11.66)**	(36.66)**

* High atmospheric temperature

** Subnormal semen quality

*** Recovered phase

to deteriorate during this month and the deterioration reached to its maximum during June where the mass activity and initial motility has been significantly lowered. The atmospheric temperature lowered down during the month of July which hastened the recovery. The recovery was slow and could be had in August month.

The other semen characteristics like color, volume and consistency were not affected by the change in atmospheric temperature.

High atmospheric temperature has been known to affect spermatogenesis and mature sperm as well (Phillips and M. Kenrie, 1934). From the present investigation it can not be made out if the

spermatogenesis is affected as the sperm concentration of most of the donated semen sample was satisfactory indicated by creamy consistency of semen. But decidedly the high ambient temperature had a deleterious effect upon mature sperm, whereby the sperm are rendered immotile. It appears that the deleterious effects of high atmospheric temperature are temporary and recovery occurs during cooler months.

Acknowledgement

We are thankful to Dr. A. L. Choudhary, Director of Research for sincere guidance and encouragement. Thanks are also due to Mr. B. P. Singh, Asstt. Fodder Agronomist for making meteorological data available for the present study.

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Prevalence Of Fungi In Bovine Semen

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ABSTRACT

A total of 113 neat semen samples from 45 bulls were examined for the presence of fungi. Approximately 50% of semen samples revealed yeasts and moulds. All the nine samples which were found negative for bacterial organisms, revealed only yeasts. *Candida* spp. was highest in proportion followed by *Mucor*, *Cryptococcus* and *Rhodotorula* spp., *Penicillium* and *Aspergillus*, *Alternaria*, *Fusarium* and *Saccharomyces* spp.

* * *

Fungi are well known as natural inhabitant of bovine reproductive tract. Some of the yeasts and moulds have been reported to be agents of mycotic abortion (Ainsworth and Austwick, 1973). Species of the genus *Candida* have been isolated from the vagina of heifers (Hajsig *et al.*, 1962) and some of these species provoke a catarrhal to purulent endometritis (Hajsig and Kopljär, 1964). Bovine semen may work as reservoir of these organisms and organisms may enter the female from contaminated semen. The present study was aimed to determine the kinds of fungi in bovine semen.

Materials and Methods

A total of 113 neat semen samples from 45 bulls were obtained from three A. I. Centres belonging to Department of Animal Husbandry, Gujarat State. Each semen sample was inoculated on

plate of Sabouraud's dextrose agar (SDA) medium. Inoculated plates were incubated at 25°C for seven days followed by further incubation of seven days before declaring a sample negative for fungi.

For identification of yeast organisms, the smear was prepared from the colony of SDA medium and subjected to Gram's staining. The cultures were sent to Mycology Department, V. P. Chest Institute, University of Delhi for detailed identification.

For mycelial fungi, individual colony was selected and examined on the basis of wet mount preparation with lactophenol cotton blue. Representative isolates of moulds were classified according to Singurd Funder (1961).

Results and Discussion

Out of 113 neat semen samples examined, 32 and 26 samples were found to be positive for yeasts and moulds respectively. All the positive samples yielded only one type of organism except two samples from which two types of yeast were isolated. The nine samples which were found negative for bacterial organism, revealed only yeasts. The species of yeasts and moulds isolated have been presented in table—1.

Approximately 50% of the semen samples examined had yeasts and moulds. Identification of yeasts revealed *Candida* spp. (19), *Cryptococcus* and *Rhodo-*

TABLE 1. Species of yeasts and moulds from bovine semen

Sr. No.	Name of species	Number isolated	Percentage
1	<i>Candida</i> spp.	19	31.67
2	<i>Cryptococcus</i> spp.	7	11.67
3	<i>Rhodotorula</i> spp.	7	11.67
4	<i>Saccharomyces</i> spp.	1	1.67
5	<i>Mucor</i>	8	13.33
6	<i>Penicillium</i>	6	10.00
7	<i>Aspergillus</i>	6	10.00
8	<i>Alternaria</i>	4	6.67
9	<i>Fusarium</i>	2	3.33
Total		60	

torula spp. (each 7) and *Saccharomyces* spp. (1). The presence of yeasts has been reported earlier in bull semen by Dholakia *et al.* (1970) and Brown *et al.* (1974). On the other hand, Richard *et al.* (1976) reported isolation of yeasts from diluted semen but not from neat semen. Highest proportion of *Candida* spp. in the present study has significance as some of *Candida* spp. have been reported to provoke endometritis (Hajsig and Kopljar, 1964) and to be agents of mycotic abortion (Ainsworth and Austwick, 1973). Kodagali (1979) has also reported *Candida* spp. from buffalo bull semen with lowered reproductive performance.

Types of moulds encountered were *Mucor* (8), *Penicillium* and *Aspergillus* (each 6), *Alternaria* (4) and *Fusarium* (2).

Zvereva and Repko (1968) and Rob and Toman (1970) have also recorded similar species of moulds in bull semen. Further, Rob and Toman (1970) drew utmost attention to disorders of reproduction particularly due to viability of moulds in deep frozen semen.

Acknowledgement

Authors are thankful to Dr. Mahendra Pal, V. P. Chest Institute, Delhi for his timely effected identification of yeast isolates. Authors are also thankful to Dr. T. N. Vaishnav, Ex. Director of Animal Husbandry, Gujarat State for giving permission for collection of materials and Dr. M. R. Patel, Dean, Faculty of Veterinary Science and Animal Husbandry, G.A.U. for providing facilities.

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CASE REPORTS

IJAR 6: : 102-104, 1985

Diplopagus Sternopagus Monster In An Indian Water Buffalo (*Bubalus bubalis*)

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ABSTRACT

Diplopagus sternopagus monsters are monozygotic conjoined twins and has not been reported in buffaloes. Monster possessed complete skeletal duplications with sternum to sternum fusion forming common thoracic and abdominal cavity and apposing each other ventrally. However, viscera appeared singly. Single hypoplastic urogenital tract could be demonstrated without discernible cervix and vagina. Hypoplasia of vulva and atresi ani was evident in twins.

* * *

Monster or "Iusus naturae" is an anomalous creature (Craig, 1930). Reports on incidence of monstrosities in buffaloes are scarce (Rai *et al.*, 1975; Bugalia *et al.*, 1980). Diplopagus sternopagus monsters are not uncommon in bovines but are rare in other domestic species (Roberts, 1971). Dystocia is most common sequelae of monstrosities in bovines (Parkinson, 1974; Bugalia *et al.*, 1980, 1982).

The present report records a case of diplopagus sternopagus monster in an Indian water buffalo (*Bubalus bubalis*).

HISTORY, CLINICAL EXAMINATION AND OBSTETRICAL OPERATIONS

A multigravida buffalo at full term

pregnancy and in acute labour was presented to Veterinary College Clinics, Ludhiana, India. Vaginal examination revealed presence of a pair of hind limbs and a tail of a dead fetus. Fetal viscera were palpable in the birth passage.

The obstetrical manipulations evidenced another pair of hind limbs and a tail in birth canal. Thus, presence of twins was confirmed. The obstetrical operations to deliver either of the fetuses proved unsuccessful. Fetotomy was considered unsuitable in the present case. Parkinson (1974) and Bugalia *et al.* (1980, 1982) also reported obstetrical manipulations and fetotomy were of limited value in bovine dystocia due to monstrosities. Hence, caesariotomy was performed and a conjoined twin monster was delivered.

Results

General description

The conjoined twin monster was delivered dead and weighed 56 kg. There was no evidence of gross tissue degeneration and mummification. Skeletal ankylosis was not noticed. Each of the twins possessed a cranium, a complete spinal column, fore limbs, hind limbs and a tail. Twins were conjoined at thoracic region in sternum to sternum position and

apposed each other ventrally (Fig. 1). Ventral opposition of crania appeared due to sternal fusion (Fig 2).

Internal organs

The common thoracic cavity of twins formed due to sternum to sternum fusion enclosed one heart, two lungs, one esophagus and a common intact diaphragm. Common abdominal wall was incomplete in prepubic region resulting in eventration of abdominal viscera through it (Fig. 3). As mentioned earlier, fetal viscera were palpable during obstetrical examination. The liver, spleen, pancreas, gall bladder and intestines appeared singly. Atresia ani was recorded in twins.

Single female genito-urinary tract was complete but hypoplastic. Genito-urinary tract comprised of two kidneys, two ovaries, two oviducts and two uterine horns without discernible cervix and vagina. Hypoplasia of vulva was noticed in twins. Both ureters terminated into urachus-like structure.

Discussion

The monster confirmed typical morphology of diplopagus sternopagus as per classification of Potter (1961) cited Roberts (1971). However, Bowen (1966) recorded cervical aplasia in a bovine double monster with sternal fusion. Roberts (1971) mentioned duplication of internal organs in such monstrosities. Furthermore, Arey (1966) stated that duplication of internal organs varies with intimacy of fusion in twins.

The incidence of diplopagus sternopagus monster is reported to be 0.00001 per cent in bovines (Hancock, 1954; Arthur, 1956). Incomplete twinning occurs due to embryonic malformations associated with monster development



Fig. 1. Diplopagus sternopagus monster showing skeletal duplications and sternal fusion.



Fig. 2. Ventral opposition of crania due to sternum to sternum fusion.



Fig. 3. Eventration of abdominal viscera from incomplete abdominal wall at prepubic region.

(Bowen, 1966; Sethi, 1967; Barrow, 1971). Present case appears to be sequelae of incomplete twinning.

Acknowledgement

Authors would like to thank Mr. C.P. Kapoor for photographic assistance.

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Induction Of Ovulatory Oestrus In A Dobermann Bitch

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ABSTRACT

Successful ovulatory oestrus and pregnancy could be induced in an infertile Dobermann bitch with the administration of Clomiphene citrate (Fertivet, FVT-300, AR-EX Laboratory, Bombay).

* * *

Pituitary gonadotrophins regulate follicular growth, maturity and ovulation. Defective ovulatory processes are manifested as delayed ovulation or failure of ovulation. Also, close co-ordination between the time of service and ovulation is essential. A case of infertility in a bitch probably of ovulatory disorder was met with and the same has been presented here.

CASE HISTORY

A healthy 3½ year old Dobermann bitch "Mona Teena," registered with the Indian National Kennel Club vide No. 15574 and reared at Baroda (Gujarat) was presented to the Veterinary College Clinic, Anand. The history showed that the bitch was a daughter of Dobermann sire "Werner Von Liebestram" and dam "Brenda", bred by Maharaja Rana Harishchandrasinghji of Jambhghoda. The present bitch "Mona Teena" was mated regularly with a highly fertile proven champion Dobermann stud "Ch. Bluntern Sans Craintes Fire Blaze," imported from Canada and registered in Kennel Club of India, during her

first four seasons/oestruses but with no conceptions. During her fifth season, she was mated with the son of previous stud which also resulted infertile. Hence the animal was brought to Veterinary College Clinic, Anand for the treatment and advise.

CLINICAL EXAMINATION, DIAGNOSIS AND TREATMENT

The bitch when presented, appeared quite healthy, normal and in very good body condition. Vaginal inspection through a sterilized speculum did not reveal any abnormality and was not in season/oestrus. However, from the history of failure of conceptions even after regular normal oestruses and breeding every time with a highly fertile stud, the condition was diagnosed as a case of ovulatory failure/disturbance resulting into infertility.

Therefore, the animal owner was advised to give her Clomiphene citrate (Fertivet, FVT-300, AR-EX Laboratory, Bombay) ¼th tablet (75 mg) orally on the 9th day and ¼th on the 11th day of her next season. Accordingly, the drug was administered orally with food during her sixth season and she was mated with Dobermann stud "Superman," a son of stud previously mated to her, on 2nd and 4th May, 1984 during the same season.



Fig. 1 Princes Mona Teena and Prince Montu of Baroda

Results and Discussion

On completion of normal gestation

period of 61 days, the bitch had a normal delivery on 5th July, 1984 and a healthy male pup was delivered (Fig. 1). The pup was allowed to suckle his mother's milk for one month. The pup was active, growing well and at 5 weeks of age, he weighed 5.5 kg with a height of 12 inches.

Clomiphene citrate has been known as a choice drug for treatment of ovulatory disturbances (Gemzell, 1975). As the present bitch was diagnosed as a case of delayed ovulation or anovulation, the same was treated with Clomiphene citrate during her subsequent season/oestrus. By this treatment, successful ovulatory oestrus and pregnancy could be induced in an infertile Dobermann bitch.

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THESIS ABSTRACT

IJAR 6: 2: 107-110, 1985

Diagnostic And Investigative Andrology In Cross-Bred Bulls M.V.Sc. Thesis

STUDENT: DR. K. V. PATEL

GUIDE: PROF: S. B. KODAGALI

A study on "Andrological investigation of nine cross-bred bulls" was undertaken during a period of one year covering cold (Nov.-Feb.), hot (March-June) and wet (July-Oct.) seasons. This included evaluation of seminal characters, assay of seminal biochemical constituents and estimation of haematological parameters.

The ranking of the bulls was made based on sperm motility, sperm concentration, abnormal sperm per cent and initial fructose concentration. Then, bulls could be grouped into ESF bulls (3), NSF bulls (4) and problem bulls (2).

The clinical observations revealed the mean scrotal circumference to be 34.93 ± 0.72 cm, testicular volume 1037.59 ± 47.85 ml, R-test 1164.81 ± 00.81 and sperm survivability test 1.96 ± 0.20 in the cross-bred bulls.

The seminal microflora in normal bulls gave the total bacterial loads within the normal limits (upto 10,000). In the problem bulls, *Pseudomonas aeruginosa* was isolated in Rosy 8-14 and haemolytic gram negative in Deolo.

Based on a total of 192 semen ejaculates, the mean normal seminal characters observed and defined were: libido 2.24 ± 0.04 , thrust 1.91 ± 0.02 , reaction time 135.00 ± 9.41 seconds, ejaculate volume 7.09 ± 0.20 ml, colour and consistency 4.44 ± 0.06 , mass motility 3.68 ± 0.08 , seminal pH 6.73 ± 0.01 , optical density

0.26 ± 0.01 , sperm concentration per ml $1121.77 \pm 36.38 \times 10^6$, sperm concentration per ejaculate $8208.17 \pm 500.46 \times 10^6$, individual motility $80.51 \pm 1.49\%$, abnormal sperm $5.76 \pm 0.37\%$, live sperm $87.70 \pm 0.81\%$, dead sperm $12.25 \pm 0.81\%$, live and dead sperm due to cold shock test $56.37 \pm 1.10\%$, and $43.51 \pm 1.09\%$, live and dead sperm due to hot shock test $75.17 \pm 1.18\%$ and $24.76 \pm 1.18\%$ and crenellation pattern 2.16 ± 0.05 .

The assay of seminal biochemical constituents was based on 192 semen/seminal plasma samples. The mean values were: initial fructose 540.59 ± 19.38 mg%, citric acid 386.73 ± 76.83 mg%, total, free and bound sialic acid 53.30 ± 1.31 , 3.90 ± 0.24 and 49.37 ± 1.31 mg%, total protein 10.29 ± 0.13 g% total solids $12.61 \pm 0.20\%$, macro-minerals such as calcium 45.68 ± 0.91 mg%, inorganic phosphorus 14.59 ± 0.45 mg%, magnesium 3.85 ± 0.11 mg%, sodium 148.12 ± 1.91 mEq/L and potassium 20.24 ± 5.49 mEq/L.

Minerals assay in cross-bred bulls semen (dry wt %) through EDAX, results were: calcium (Ca) 4.058 ± 0.718 , phosphorus (P) 12.836 ± 2.428 , Ca:P ratio 0.397 ± 0.096 , sodium (Na) 6.460 ± 1.200 , potassium (K) 15.036 ± 2.982 , Na:K ratio 0.627 ± 0.181 , sulphur (S) 28.727 ± 1.960 , chloride (Cl) 22.240 ± 1.014 , copper (Cu) 7.821 ± 1.089 and zinc (Zn) 3.394 ± 0.463 .

Estimation of haematological parameters in crossbred bulls based on 48 blood samples, the results were: haemoglobin (Hb) 12.27 ± 0.22 g%, packed cell volume (PCV) 36.81 ± 0.69 %, erythrocyte sedimentation rate (ESR) 6.83 ± 0.47 mm/24 hrs, total erythrocyte counts (TEC) $7.31 \pm 0.23 \times 10^6$ /cumm, total leucocyte counts (TLC) 7514.79 ± 277.90 WBCs/cumm, differential leucocyte counts (DLC) per cent includes neutrophils (N) per cent 41.88 ± 1.69 , eosinophils (E) per cent 2.50 ± 0.25 , basophils (B) per cent 0.00 , lymphocytes (L) per cent 53.85 ± 1.71 , monocytes (M) per cent 1.75 ± 0.22 ; mean corpuscular volume (MCV) 53.05 ± 2.25 cubic micron, mean corpuscular haemoglobin (MCH) 17.46 ± 0.54 μ g, mean corpuscular haemoglobin concentration (MCHC) 34.47 ± 1.00 %, plasma protein 7.66 ± 0.13 g% and blood glucose 52.21 ± 1.86 mg%.

The 'F' test analysis for the effect of bulls, effect of seasons and effect of bulls \times seasons interaction for the various seminal attributes, biochemical constituents and haematological parameters revealed the effect to be highly significant:

- (1) in seminal characters of bulls for libido, thrust, colour and consistency, mass motility, seminal pH, optical density, individual motility, abnormal sperm per cent, live and dead sperm per cent due to hot shock test and crenellation pattern;
- (2) in biochemical constituents for total protein, total solids and magnesium and
- (3) in haematological parameters for Hb, PCV, TEC, E%, B%, M% and plasma protein.

The bull effect was significant:

- (1) in seminal characters for ejaculate volume and live sperm per cent due to cold shock test,

(2) in biochemical constituents only for potassium and

(3) in haematological parameters for MCH. In the remaining parameters the bull effect was not significant.

The 'F' test did not show any seasonal effects with regards to seminal characters and biochemical constituents. However, the effect of seasons on haematological parameter was highly significant for ESR, N%, L%, MCV, MCH and blood glucose and significant for PCV and M%.

The effect of bulls \times seasons interaction was highly significant:

- (1) in seminal characters for libido, colour and consistency, seminal pH and crenellation pattern,
- (2) in biochemical constituents only for magnesium and
- (3) in none of haematological parameters.

The interaction effect was significant in semen characters for thrust, reaction time, mass motility and optical density. The interaction effects were non-significant in biochemical constituents and haematological parameters.

The correlation studies were made within seminal characters, biochemical constituents and haematological parameters and between seminal characters and biochemical constituents.

In semen characters: libido was correlated with thrust ($r = +0.256$), reaction time ($r = -0.487$), colour and consistency ($r = -0.144$), optical density ($r = -0.153$), sperm concentration per ml ($r = -0.190$); thrust was correlated with reaction time ($r = -0.363$); reaction time was correlated with ejaculate volume ($r = +0.178$), mass motility ($r = +0.152$), individual motility ($r = +0.172$); ejaculate volume was correlated with colour and consistency ($r =$

+0.156), seminal pH ($r = -0.148$), sperm concentration per ejaculate ($r = +0.489$); colour and consistency was correlated with mass motility ($r = +0.490$), seminal pH ($r = +0.173$), optical density ($r = +0.425$), sperm concentration per ml ($r = +0.485$), sperm concentration per ejaculate ($r = +0.243$), individual motility ($r = +0.345$), crenellation pattern ($r = +0.398$); mass motility was correlated with optical density ($r = +0.395$), sperm concentration per ml ($r = +0.454$), sperm concentration per ejaculate ($r = +0.236$), individual motility ($r = +0.640$), abnormal sperm per cent ($r = -0.302$), live and dead sperm per cent ($r = +0.257$) and ($r = -0.254$), live and dead sperm per cent due to cold shock test ($r = +0.208$) and ($r = -0.209$), live and dead sperm per cent due to hot shock test ($r = +0.352$) and ($r = -0.352$), crenellation pattern ($r = +0.363$); seminal pH was correlated with optical density ($r = +0.170$), sperm concentration per ml ($r = +0.201$); optical density was correlated with sperm concentration per ml ($r = +0.940$), sperm concentration per ejaculate ($r = +0.426$), individual motility ($r = +0.241$), crenellation pattern ($r = +0.436$); sperm concentration per ml was correlated with sperm concentration per ejaculate ($r = +0.435$), individual motility ($r = +0.253$), dead sperm due to cold shock ($r = -0.144$), crenellation pattern ($r = +0.475$); sperm concentration per ejaculate was correlated with individual motility ($r = +0.188$), live and dead sperm due to hot shock test ($r = +0.147$) and ($r = -0.142$), crenellation pattern ($r = +0.249$); individual motility was correlated with abnormal sperm per cent ($r = -0.408$), live and dead sperm per cent ($r = +0.383$) and ($r = -0.381$), live and dead sperm per cent due to cold shock test ($r = +0.178$) and ($r = -0.182$),

live and dead sperm per cent due to hot shock test ($r = +0.396$) and ($r = -0.387$), crenellation pattern ($r = +0.186$); abnormal sperm per cent was correlated with live and dead sperm per cent ($r = -0.568$) and ($r = +0.568$); live and dead sperm per cent due to cold shock test ($r = -0.228$) and ($r = +0.229$), live and dead sperm per cent due to hot shock test ($r = -0.460$) and ($r = +0.455$); dead sperm per cent was correlated with live sperm per cent ($r = -0.998$) live and dead sperm per cent due to cold shock test ($r = -0.469$) and ($r = +0.472$), live and dead sperm per cent due to hot shock test ($r = -0.567$) and ($r = +0.580$); live sperm per cent was correlated with live and dead sperm per cent due to cold shock test ($r = +0.467$) and ($r = -0.470$), live and dead sperm per cent due to hot shock test ($r = +0.563$) and ($r = -0.576$); dead sperm per cent due to cold shock was correlated with live sperm per cent due to cold shock test ($r = -0.995$), live and dead sperm per cent due to hot shock test ($r = -0.419$) and ($r = +0.431$), crenellation pattern ($r = -0.140$); live sperm per cent due to cold shock test was correlated with live and dead sperm per cent due to hot shock test ($r = +0.415$) and ($r = -0.427$), crenellation pattern ($r = +0.141$) and dead sperm per cent due to hot shock test was correlated with live sperm per cent due to hot shock test ($r = -0.995$).

In seminal biochemical constituents: initial fructose was correlated with free sialic acid ($r = -0.238$), total protein ($r = +0.143$), calcium ($r = -0.161$), inorganic phosphorus ($r = -0.234$); citric acid was correlated with total and bound sialic acid ($r = +0.203$) and ($r = +0.196$), total protein ($r = +0.153$), total solids ($r = +0.281$); total sialic acid was correlated with bound sialic acid

($r=+0.981$); total solids was correlated with total protein ($r=+0.393$), calcium ($r=+0.221$), potassium ($r=-0.234$) and sodium was correlated with potassium ($r=+0.488$).

The correlation studies between seminal characters and biochemical constituents revealed: initial fructose was correlated with sperm concentration per ml ($r=+0.147$); citric acid was correlated with sperm concentration per ml ($r=+0.215$); total sialic acid was correlated with ejaculate volume ($r=+0.184$), abnormal sperm per cent ($r=+0.238$), live sperm per cent ($r=-0.212$); total solids was correlated with mass motility ($r=+0.283$); inorganic phosphorus was correlated with live sperm per cent ($r=-0.228$); sodium was correlated with seminal pH ($r=+0.180$) and potassium was correlated with live sperm per cent ($r=-0.163$).

Out of nine bulls studied two bulls (22.22%) were problem bulls. On detailed clinical andrological examination, seminal, biochemical and histopathological studies these bulls were diagnosed to have testicular hypoplasia.

The clinical findings in normal and problem bulls for comparison were: scrotal circumference to be (34.93 ± 0.72 vs 30.75 ± 0.51 cm), testicular volume (1037.59 ± 47.65 vs 837.50 ± 23.72 ml), R-test (1164.81 ± 100.81 vs 425.00 ± 17.68) and sperm survivability test in cervical mucus (1.96 ± 0.20 vs 0.84 ± 0.21), respectively.

The comparison between normal and problem bulls:

(1) in seminal characters were: sperm motility (3.68 ± 0.08 vs 1.43 ± 0.13),

sperm concentration/ml (1121.77 ± 36.58 vs $653.75\pm90.48\times10^6$), abnormal sperm per cent (5.76 ± 0.37 vs 28.42 ± 7.52) and live sperm per cent (87.70 ± 0.81 vs 38.73 ± 0.37);

(2) in biochemical constituents levels were: initial fructose (540.59 ± 19.38 vs 393.18 ± 95.58 mg%), citric acid (386.73 ± 76.83 vs 352.86 ± 29.84 mg%), total sialic acid (53.30 ± 1.31 vs 39.95 ± 7.44 mg%), total protein (10.29 ± 0.13 vs 8.03 ± 0.29 g%), total solids (12.61 ± 0.20 vs $7.68\pm0.35\%$) and inorganic phosphorus (14.59 ± 0.45 vs 14.14 ± 3.27 mg%);

(3) in minerals assay through EDAX (dry wt %), the results were: Ca:P ratio (0.397 vs 0.822), Na:K ratio (0.627 vs 0.345), sulphur (28.727 vs 15.509), chloride (22.240 vs 42.104), copper (7.821 vs 4.380) and zinc (3.394 vs 1.567); respectively.

Problem bull Rosy 8-14 had low germinal cell resistance hypoplasia and subsequent testicular degeneration and the bull Deolo having bilateral total testicular hypoplasia as confirmed on histopathological examinations.

The information regarding sexual function in cross-bred bulls is scarce. Hence, the present study on andrological investigation of cross-bred bulls was undertaken and this revealed that the clinical, seminal, and biochemical studies proved as aid to assess and diagnose the sexual functions of the bulls. Differences could be noticed between normal and problem bulls.

Ph.D. Thesis

"Comparative study of sexual behaviour and semen characteristics of young Kankrej \times Jersey (F_1) and Kankrej \times Holstein Friesian (F_1) bulls in relation to weather factors."

Student: G.P. Bhagoji

Major Advisor: Dr. A.D. Dave

Minor Advisor Dr. S.B. Kodagali

Gujarat College of Veterinary Science
and Animal Husbandry, Gujarat
Agricultural University, Anand Campus, Anand.

A study was undertaken at the Gujarat College of Veterinary Science and Animal Husbandry, Anand from 1-11-1982 to 31-10-1983, to ascertain and compare the effect of weather factors on sexual behaviour and semen characteristics of young Kankrej \times Jersey (F_1) and Kankrej \times Holstein Friesian (F_1) bulls.

The maximum and minimum temperatures inside the shed ranged from 24.43°C to 37.64°C and 14.14°C to 28.14°C respectively.

The Jersey (4.074 ± 1.240 min.) and the Holstein (3.859 ± 0.806 min.) crosses did not differ in the reaction time, the former being affected by season ($P < 0.05$). The ambient temperature and relative humidity showed respectively positive and negative association with the reaction time of Holstein and Jersey crosses.

The mating technique of all the bulls of both the breed groups was normal.

The average volume of two ejaculates was $5.385 + 1.429$ and 6.126 ± 0.835 ml for Jersey and Holstein crosses respectively, the difference being significant ($P < 0.01$). Season affected ($P < 0.05$) the ejaculate volume of Jersey crosses. It was significantly associated with maximum, minimum and mean temperature and THI in Holstein crosses.

The mass activity of the semen ejaculates of Holstein crosses was superior ($P < 0.01$) (3.280 ± 0.381) to that of Jersey (3.004 ± 0.453) crosses and was positively associated ($P < 0.05$) with THI.

The initial motility score of semen ejaculates of Holstein crosses was higher ($P < 0.01$) (4.131 ± 0.412) than that of Jersey crosses (3.910 ± 0.454); that of the latter being affected by summer, and the former by relative humidity.

The Holstein crosses had higher ($P < 0.01$) sperm concentration (982.631 ± 166.403 million/ml) and also produced more ($P < 0.01$) sperm per ejaculate ($6,077.090 \pm 1,347.707$ million/ejaculate) than the Jersey crosses (809.658 ± 166.403 million/ml and $4,274.132 \pm 1,106.817$ million/ejaculate). The total sperm production was positively associated with minimum temperature, mean temperature, RH and THI in case of Holstein crosses.

The proportion of abnormal sperms was less ($P < 0.01$) in the ejaculates of Holstein crosses (8.667 ± 3.038 per cent) than in that of the Jersey crosses (10.765 ± 3.850 per cent); being higher ($P < 0.05$) in summer and monsoon respectively in the two breed

groups. In both the breed groups, it was associated with minimum and mean temperatures, RH and THI. In Holstein crosses it was associated with maximum temperature also.

The semen pH of Jersey (6.692 ± 0.301) and Holstein (6.679 ± 0.273) crosses was significantly associated with relative humidity and maximum temperature.

The weekly gain in weight (Jersey 2.885 ± 2.064 kg, Holstein 3.413 ± 1.735 kg) and the average dry matter consumption per 100 kg body weight (Jersey 2.694 ± 0.122 kg, Holstein 2.678 ± 0.114 kg) of both the groups were affected by season.

ISSAR NEWS

Item: 1

An emergency meeting of the organising committee was held on 10th and 11th Dec. 1985 in the Dept. of Animal Reproduction at 11 a.m. when following members were present.

Dr. B.R. Deshpande, Dr. A.S. Kaikini, Dr. D.P. Velhankar, Dr. S.B. Kodagali, Dr. C.R. Sane, Dr. V.B. Hukeri, Dr. V.L. Deopurkar, Dr. S.A. Sonawane and Dr. S.R. Chinchkar.

Item: 1 The inaugural and the Scientific session programme for the First Asian Congress on Animal Reproduction to be held on 11th, 12th & 13th Dec. 1985 was chalked out and approved. It is gratifying to note that His Excellency the Governor of Maharashtra Shri K. Prabhakar Rao has kindly consented to be the chief guest for inaugural function. Dr. Manibhai Desai has kindly accepted to preside.

Item: 2 148 abstracts received for presentation at the congress were suitably classified session and subject wise and it was resolved to publish them in the souvenir issue of the IJAR.

Item: 3 Various matters Concerning accomodation transport, hospitality etc were discussed.

Item: 4

To All Concerned.

Subject: The Indian Journal of Animal Reproduction
a bi-annual Scientific journal
Request for beneficiaries

Sir,

The Indian Journal of Animal Reproduction is the official organ of the Indian Society for the Study of Animal Reproduction (ISSAR) and is being regularly published biannually since 1981. The object is to disseminate the recent advances in education, research and extension in the field of Animal Reproduction. This is the only journal of its kind in the country which is recognised by the Indian Council of Agril. Research, New Delhi and is being abstracted by the Commonwealth Agricultural Bureau, U.K. & others.

ISSAR is entirely depending on advertisements at present which is an uncertain, resource. The amount collected does not commensurate with the cost involved in publishing the journal.

For publishing 1000 copies bio-annually the ISSAR is incurring an expenditure on an average Rs. 40,000/-. The professional expertise involved in the production of this journal is honorary and hence not accounted for in this Rs. 40,000/-. Considering the rise in the cost of material required for publication the ISSAR has now thought of seeking for beneficiaries for putting the journal on no profit no loss basis. We would therefore be interested to know whether you can participate in this project of National interest.

1. Would you be willing to be considered as a full sponsorer of this journal for a minimum period of three years by contributing actual cost of production?

2. Whether you would like to be considered as one of the beneficiaries by contributing Rs. 5000/- for three years period.

Due acknowledgement will be made in the journal of sponsorers or co-sponsorers.

(Dr. B.R. Deshpande)
President, I.S.S.A.R.

Item: 5

Best Veterinary Academician Award to Dr. C.R. Sane

The best Veterinary academician award by Poonawala Group of Organisations in its first year of inception was presented to Dr. C.R. Sane, Patron, ISSAR on 31-7-85 by Dr. S.S. Mehendale, president of Veterinarians' Association, K.K.V. Bombay-12.

Item: 6 ISSAR Gujarat Chapter: O.No. CDS/KVS/7523-51 1984

Directorate of Animal Husbandry,
Gujarat State,
Krishi Bhavan,
Paldi, Ahmedabad-6
29-9-84

Circular Letter

Concerned officers of this Dept. are hereby informed that the Indian Society for Study of Animal Reproduction publishes Indian Journal of Animal Reproduction, a biannual publication. This contains recent information and development in animal reproduction. This is of great utility in A.I. Centres, Semen Banks, Cattle Breeding Farms and Veterinary Dispensaries.

The annual subscription of this Journal is Re. 50/-. The Editor of the Journal is Dr. S.B. Kodagali, Professor of Gynaecology, Veterinary College, Anand. It is recommended that you may contact him directly and subscribe the Journal.

Sd. Hari Singh Pannu
Director
Dept. of Animal Husbandry
Gujarat State, Ahmedabad

It is desired that the Executive Committee members of the ISSAR chapters in different States will contact the Directorate of Animal Husbandry in their State and request for issue of such circular so that the ISSAR can be strengthened vis-a-vis IJAR.

I.M. Shah
E.C.M.
ISSAR
Gujarat Chapter

Item No. 7**List of Life Members**

Sr. No.	Reg. No.	Name and address
70	96	Dr. S.A. Sonawane A/8, Madhur Co-op. Hsg. Soc. Chincholi, Bund Road Malad (West) Bombay-64

List of life members ISSAR ctd.

71	475	Dr. Sayed Abdul Quayam Professor and Head, Department of Obst. & Gynaec Madras Veterinary College Madras-7.
72	513	Dr. Dharamveer Bishnoi A.P.O. I/c Mobile Unit III, Urmul-Laonkaransar Bikaner—Rajasthan
73	205	Dr. F.S. Kavani Associate Professor, College of Veterinary Science & A.H. Gujrath Agricultural University, Datiwada, Dist: Palanpur, Gujrath
74	198	Dr. A.H. Chaudhary Department of Gynaecology, College of Veterinary Science Assam Agricultural University Kanapara, Gauhati—781 022 Assam State

OBITUARY

Biodata of Dr. P. Bhattacharya

It is with great measure of shock to learn the untimely passing away on 21st August, 1985 of Late Dr. P. Bhattacharya former Animal Husbandry Commissioner to the Govt. of India and member, National Commission on Agriculture.

Dr. P. Bhattacharya was born on 15th November, 1910. He had his B.Sc. (Hons) and MSc degree in Zoology from the Calcutta University. He went to UK and obtained Ph.D. degree in Animal Genetics from Edinburgh University.

Dr. P. Bhattacharya was Head, Division of Animal Genetics, IVRI Izatnagar. Credit goes to him for the large scale field application of A.I. for breeding & development of dairy cattle and buffaloes. He was associated with many National and international professional organisations in varied capacities. He has great contributions in the scientific books published in India, USA and UK. He was member and subsequently chairman of the Panel on Animal Breeding since 1954. He was President of the Indian Dairy Association from 1958-1972. He is the founder President of the Indian Association for Animal Production and Indian Society of Animal Genetics and Breeding.

In his passing away the country has lost a distinguished and illustrious scientist who will long be remembered in the cause of Animal breeding and development, Agricultural research and education in India and abroad.

The members of ISSAR share the sorrow and pay their homage
May his soul rest in Peace.

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**THE INDIAN JOURNAL OF
ANIMAL REPRODUCTION
(SOUVENIR ISSUE)**

**Official Organ of the Indian Society
for the Study of Animal Reproduction**

Vol. 6

No. 2

DECEMBER 1985

First Asian Congress on Animal Reproduction

Organised by:

THE INDIAN SOCIETY FOR THE STUDY OF ANIMAL REPRODUCTION

in association with

**THE INDIAN COUNCIL OF AGRICULTURAL RESEARCH & KONKAN KRISHI
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Bombay**

DECEMBER 1985, 11th, 12th, 13th

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**ABSTRACTS OF PAPERS
FOR PRESENTATION**

Dr. S.B. Kodagali
Publication Editor
Prof & Head,
Gynaecology & Obsts.
College of Vety. Sci.
G.A.U. Anand-388 001

SESSION: I

Infectious infertility and Repeat breeding

1. A clinico biochemical studies on cervicovaginal mucus of repeat breeding cattle.

R. GUPTA, R.K. PANDIT, R.A.S. CHAUHAN, M.L. PORWAL

Mhow Veterinary College

The changes in the composition of the cervical mucus make the genital tract environment unfavourable for sperm survival, leading to infertility. It has been suggested that some physical, physico-chemical and biochemical values for the cervical mucus of repeat breeding cows are changed tremendously (Pandey *et. al.*, 1983, Enkhia, *et. al.*, 1983). Sperm agglutinating factors are also found in the cervical mucus resulting into the infertility (Miyano *et. al.*, 1982).

In the present paper the physical

characteristics (Color, consistancy and transparency), rheological properties (viscosity, flow elasticity, spinbarkeit, tack value and plasticity), physiochemical properties (pH & arborization) and biochemical constituents (Drymatter, water, calcium, phosphorus, chloride and total protein) of the cervical mucus of repeat breeding cows are studied. The *invitro* effect of the cervical mucus of repeat breeding cattle on the sperms i.e. viability, penetrability and agglutination are discussed.

2. Oestrus cycle pattern in repeat breeding crossbred cattle (K×J & K×H)

G.B. MEHTA, B.K. BHAVSAR, M.N. MANSURI, J M. PATEL, S.B. KODAGALI

Gujarath Veterniary College, Anand 388001

Inter-oestral lengths of 403 oestrus cycles in 38—Kankrej×Jersey (K×J) and Kankrej Holstein (K×H) heifers and cows were studied for the effect of breed, season, age, lactational yield and parity. The normal range of oestrus cycle length (18-25 days) were observed in 52.23% and 49.19% of these animals

respectively, the incidence of short oestrus cycles (>17 days) was 7.0 and 8.54% in K×J and K×H cattle. Long cycles (26 days) were observed in 40.77 and 42.27% of K×J & K×H cattle respectively. Occurence of repeat breeding cycles was more in K×H (61.04%) compared to K×J (38.96%). Repeating

oestrus cycles were found to be more in cold months (38.95%) compared to other seasons. Repeating oestrus cycles were found to be more prevalent (62.04) % in cross bred cattle of 25-48 months age group in the present study. 69.81 % of K×J cows having milk yield below 3000 kg. K×H cows having total milk

yield above 3000 kg. showed repeat oestrus cycles in 62.09% of cows. Among the cows 63.46% and 69.06% Of the 1st lactation K×J and K×H cows showed repeat breeding. Occurrence of repeat oestrus cycles were more in K×J (56.68%) and (88.62%) K×H cows compared to heifers.

3. Studies on cervical mucus enzymes in normal and Repeat breeding cross bred cows.

V.K. SHARMA, S.S. TRIPATHI

College of Veterinary Science A.H.G.A.U.S.K. Nagar, 385506

The cervical mucus collected from normal and repeat breeding cross-bred cows was subjected to determine the concentration of various enzymes and their interrelationship during oestrus. A wide individual variation was observed in LDH concentration in both groups of the animals. The mean values of LDH, IDH, AKP AND ACP in normal and

repeat breeding cross-bred cows were 91.29 ± 12.88 & 82.13 ± 1.85 , 9.85 ± 0.33 & 9.34 ± 0.24 , 21.17 ± 2.34 & 15.87 ± 1.73 , 3.23 ± 0.54 & 3.56 ± 0.40 (in/lit.) respectively. The variation in the average values was not significant. The AKP and ACP contents were significantly positively correlated in the repeat breeding group of cows.

4. Histopathological investigations of fallopian tubes in Repeat breeding cows.

F.S. KAVANI, K.S. PRAJAPATI, S.B. KODAGALI

College of Veterinary Science & A.H. Gujraath—385506

The animals investigated in the present study included 10 cows and 14 heifers, which were chronic repeat breeders. The both fallopian tubes were collected on P.M. examination for histo-pathological study. Out of 24 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.

5. Incidence of Reproductive disorder in exotic and Indian cattle.

J.C. DUTTA, J.N. DAS, C.K. RAJKONWAR

College of Veterinary science, Khanapara-Guwahati, 781022

The incidence of certain reproductive disorders in Jersey, Red-Dane and Red Sindhi cattle under prevailing climate (hot and humid climate) of Assam was studied. Out of 1124 observations, the overall incidence of abortion, early

embryonic death, mummified foetus, dystocia, retained foetal membranes, cervico-vaginal prolapse, pyometra and post-partum metritis were found to be 9.70; 0.45; 0.09; 0.45; 3.65; 0.09; 1.78 and 1.60 per cent respectively.

6. Some Observations on treatment of Repeat breeding problem in cows and Buffaloes.

D.R. PARGAONKAR

College of Veterinary and Animal Science Parbhani-431402

73 Cases (56 Cows and 17 buffaloes) of Repeat Breeding problem were presented to Veterinary Polyclinic, College of Veterinary and Animal Sciences, Marathwada Agril. University, Parbhani. Cervical mucus swabs were subjected to Microbiological investigations and also for sensitivity tests.

It was observed that two trips of the animal during the same oestrus period

(i.e. one for A.I./R.S. and another for intra-uterine therapy) can be avoided. Intra-uterine therapy is carried out immediately on getting the animal detected in heat and A.I./R.S. can be arranged after few hours following intra-uterine therapy. Observations on response to the different therapies tried are being communicated.

7. Treatment with GnRH analogue (Buserelin) at mid luteal phase in Repeat breeding dairy cows improves the breeding efficiency.

M.R. BHOSREKAR, A.J. INAMDAR, B.M. JOSHI, Y.P. PHADNIS, B.R. MANGURKAR

B.A.I.F., Uralikachan PUNE

This study was conducted in 5 herds of dairy cows (Western India) on a total of 81 females. They were randomized and divided in two groups treated (n=44) and controls (n=37) after strict

selection conditions namely 3 consecutive failure of AI at regular intervals. The two groups had similar intervals between calving and 1st AI (73 days), same mean parity and similar and very regular mean

duration of the 4 consecutive cycles (22 days). After the 3rd A.I., one heat was observed on which no insemination took place. Then the treated cows received i.m. 20 ug of the GnRH analogue (Buserelin) and the control, a placebo (5 ml saline solution) on day 13 after the previous heat. The expected interval between injections and the following oestrus was 13 days or less.

During this period, more cows in the

treated than in the controls came in heat (33/44 vs 16/37 respectively, $P < 0.01$), the conception rates were 38% vs 12.5% ($P < 0.02$) and the mean intervals between injections and conceptions were 28.6 vs 43.3 days respectively for treated and control animals ($P < 0.05$).

It was concluded that this GnRH analogue treatment significantly contributed to improve breeding efficiency in Repeat Breeders.

8. Studies on Histopathology of uterus of metritis cases in slaughtered cows & co-relation with the Bacterial isolates.

N.C. MAZUMDAR, A.N. CHAKRABARTY, B. KANJILAL, A. CHATTERJEE
H.M. BHATTACHARYAY

Bidhan Chandra Krishi Vishwa Vidyalaya, Mohanpur

Eight hundred thirty five cows were clinically examined for presence of metritis and 200 (23.95%) cows appeared to have thickening, flabbiness, atony of uterine wall with or without discharge of varying nature.

Gross lesions of metritis were detected in 25 (12.5%) of 200 cows after slaughter and such changes were catarrhal (40%), fibrinous (36%) and suppurative (24%).

Wet and ZN stained smears of metritis lesions did not reveal presence of *T. foetus* and acid-alcohol fast organisms respectively. Gram stained smears showed presence Gram positive cocci, and Gram positive & negative bacilli. Cultural isolations yielded 52% Streptococci; 40% and 16% coagulase positive and negative Staphylococci respectively; 24% diphtheroids; 16% *P. pyocyanea*; 36% *E. coli*; 40% Proteus and 12% Anthracoides. More than one isolates were obtained from each lesion.

Histopathological examinations revealed presence of acute catarrhal (28%), acute fibrinous (12%), acute suppurative (24%) and chronic (36%) inflammatory changes in 25 tissues having gross lesions of metritis.

Inflammatory changes of uteri characterized by catarrhal (40%), fibrinous (36%) and suppurative (24%) types are considered to be responsible for infertility/sterility of cows under the study.

Streptococci, Staphylococci (both coagulase positive and negative), Corynebacterium, Pseudomonas, *E. coli*, Proteus and Anthracoids are found to be present in uteri having lesions of metritis. Multiple infection has been found to be a common feature. *T. foetus* and acid-alcohol fast organisms are not detected under the study.

Acute catarrhal (28%), fibrinous (12%) suppurative (24%) and chronic (36%) types of inflammatory changes are associated with the metritis detected under the study.

9. Postpartum changes of uterus and ovaries in relation to uterine microflora in cows.

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A study conducted on a total of 30 cows of which 20 were negative and 10 positive for uterine microflora in the first two weeks of parturition revealed that the time of involution of the cervix and uterus was 22.60 ± 1.54 and 25.60 ± 0.24 days respectively in microflora free and 28.40 ± 1.83 and 29.60 ± 1.22 days respectively in microflora positive cows. Rate of involution of the cervix and the uterus was rapid up to 16 day postpartum in both the groups. In microflora free cows lochial discharge was free flowing up to 4 days and ceased from 28 days whereas in the microflora positive group free-flowing discharge was not common

but scanty discharge was observed even up to 40 days postpartum. The blood tinged lochia became clear from 20 to 24 days in both the groups. Watery lochial discharge became thicker in consistency from 12 days onwards. The time of regression of pregnancy corpus luteum, time of development of palpable follicle and the postpartum oestrus interval were 10.45 ± 1.77 , 12.80 ± 0.79 and 59.60 ± 9.46 days respectively in microflora free and 10.00 ± 0.95 , 12.50 ± 1.44 and 53.00 ± 8.58 days respectively in microflora positive cows with no significant difference between groups.

10. Treatment of uterine infections with 'Utrovect' in Repeat breeder cows and Buffaloes.

I.M. SHAH, V.R. JANI

Directorate of A.H., Ahmedabad

29 buffaloes and 16 cows (total 45 animals) were treated with Utrovect tablets orally for uterine infections. Out of 10 cases of endometritis, 7 animals (70%) were cured and conceived. The dose given was 20, 20 and 10 tablets from 1 to 3 days. Out of 25 animals having chronic metritis, 14 animals (56%) were cured and conceived. The dose given was 30, 30 20 and 20 tablets from

1 to 4 days. And out of 10 animals having pureperal metritis, 6 animals (60%) were cured and conceived. The dose given was 40.40 and 20 tablets from 1 to 3 days. The treatment was repeated in necessary cases. The number of services required for conception were from 1 to 3, 1 to 4 and 1 to 4 in endometritis, chronic metritis and pureperal metritis respectively.

11. Comparative efficacy of drugs to reduce the occurrence of repeat breeding in rural dairy animals.

G.C. JAIN

National Dairy Research Institute, Karnal, Haryana, 132001

To minimise the incidence of repeat breeding in dairy animals, various drugs were administered in 329 repeat breeding dairy animals with pathological and nonpathological genital conditions. Out of this population, 105 repeat breeders with mild/sub clinical non specific pathological conditions were tried with the intra uterine administration of liquid terramycin solution, Mastalone-U, Benzyle penicillin G Sodium and 0.5% lugols solution. The response to different treatments was measured as the effect on conception with in 42 days of administration (2 oestrous cycles). The response of 0.5% lugols iodine solution was observed most effective (67%) followed by liquid terramycin and Benzyle Pencillin G-Sodium solution (52-53%) and Mastalone—U (41%).

The other group of 224 animals having no history of genital infections, were administered progesterone, HCG and oxytocin intra-muscular along with insemination. The response on conception was found most effective with 125 mg progesterone (68%) followed by HCG (65%) and oxytocin (64%). The study suggested that the incidence of repeat breeding in dairy animals can be lowered with the administration of lugols solution and crystalline penicillin G Sodium solution in animals with mild genital infection whereas the incidence can be controlled with the administration of progesterone (125 mg) and HCG in animal having repeated for more than four times. Further trials are needed to substantiate these findings.

12. Spermatozoal agglutination in semen and cervical mucus of repeat breeding cows.

A.B. GADGE, V.L. DEOPURKAR, B.R. DESHPANDE, B.V. JALNAPURKAR, P.M. PUNTAMBEKAR

Six Repeat breeding cows of Gir × HF cross and Gir × Jersey cross with a history of minimum seven inseminations were selected for the study. All these 12 animals had no palpable abnormality in genitalia and were free from genital infections. Six normal (cycling) cows and 6 Heifers maintained on the same farm were used as control. Blood serum and the cervical mucus of all the above cows were collected. Semen samples of the bulls from the respective crosses, which were commonly used on the farm for insemination were collected to carry out agglutination test with blood serum and

cervical mucus.

The agglutination titres in case of repeat breeders were found to be higher as compared to that of control. None of the twelve animals from control showed serum and cervical mucus agglutination titre of more than 1:20. A titre of 1:40 or more was observed in eight and seven out of 12 repeat breeders in serum and cervical mucus respectively.

The maximum titre obtained in one of the repeat breeders was 1:1280 for serum whereas titre of the same animal for cervical mucus was found to be 1:640.

SESSION: II

Enhancing Reproductive Potential

1. Enhancing reproductive potential of Farm animals.

R.T. KULKARNI

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Reproductive potential of Indigenous animlas is far from satisfactory, in organised Dairy Farm by proper breeding, Feeding and management considerable improvement has taken place during last 20 to 30 years. However in India present breedable cow population is estimated at 50 millions and of buffalo (above three years of age) at 25 million. A systematic efforts are required by training man-power, by producing and importing high quality semen for breeding, better feeding and management.

Action for food production (AFPRO) a Delhi based secular non-governmental voluntary organisation of Christian inspiration is co-ordinating, supporting and providing technical assistance in cattle improvement programmes since 1966.

AFPRO was instrumental to obtain some 22,000 doses of Frozen Semen for BAIF—Bharatiya Agro Industries Foundation, Urulikanchan in 1970 through WRSO, Newzealand and Christian Aid London.

AFPRO has done a pioneering work

in the field of cattle improvement by importing exotic bulls and heifers through vol-agencies mainly through the Australian Society "For Those Who Have Less", the American Society "Heifer Project International" and the British Society "Christian Aid: These cattle were mainly donated to Government of India and State Government, Military Farms and represent the basic stock on which a major part of the earlier efforts of the state were based for upgrading the local cattle. From 1966 to 1978 total 1046 cattles were imported throug AFPRO.

To increase the reproductive potential of indigenous non-descript animals AFPRO is assisting grass-root voluntary organisations in importing A.I. equipments, establishment of A.I. Centres, training of man-power and implementing a dairy programme.

Recently AFPRO was instrumental in importing "top quality Frozen Semen" from Milk Marketing Board, UK" for BAIF which is being used to breed seed stock to get good herd for further National development.

2. Fertility following Heterospermic inseminations in Surti Buffaloes

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Panchmahal Dist. Co-op. Milk Producers, Godhra-389001 and
Veterinary College, Anand.

In the field level fertility trials in Surti breed of buffalo, the frozen semen from four different Surti bulls (B1, B2, B3, & 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—291 buffaloes/heifers were inseminated, out of that 238 were followed 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.

3. Effect of season of calving on various economic characters in Bhadavwair Buffaloes.

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The effect of season of calving on various economic traits were tested by analysis of variance and found that the variance ratio between season of calving and all economic traits were positive and non significant except between season of calving and lactation length and service period which were significant. As regards the average lactation milk yield in respect of season, Vasant ritu calvers produced higher milk (775.21 kg) followed by Versha ritu, Hemant ritu, Sharad ritu and Greesham ritu calvers but the lactation length was longer in Vasant ritu calvers. In respect of other seasons. Only slight differences in mean value of gestation period were observed in all seasons which was statistically non significant.

Average value of intercalving period

in respect of season indicated that the Vasant ritu calvers had slightly longer time than Versha, Hemant, Sharad and Greesham ritu calvers respectively and dry period was found to be longer in Hemant ritu calvers followed by Versha, Sharad, Vasant and Greesham ritu calvers. The average service period in respect of season of calving was found to be 155.8 days (in Varsha ritu); 77.5 days (in Sharad ritu); 61.6 days (Hemant ritu) and 43.0 days (Greesham ritu) calvers respectively. The mean value of total fat and total solid yield in respect of season, indicated that the Vasant ritu calvers had high fat yield as well as total solid yield followed by Versha, Sharad, Hemant and Greesham ritu calvers respectively.

4. Studies on some economic traits related to milk production in cows of different Genetic groups.

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C.S.A. University of Agriculture & Technology, Kanpur 208002

The present research project was carried out to evaluate variability among different cross bred cows of different genetic combinations, developed Holstein Friesian, Jersey and Sahiwal for different economic traits related to milk productivity. The six different genetic groups cross bred female calves were taken according to their parental combination.

Analysis of variance for all the genetic groups for different economic traits were worked out and the finding was that all the genetic groups had no significant differences among themselves except for the lactation milk yield. The average birth weight of female calves were found to be highest in $3/8$ HF + $3/8$ S (23.00 + 2.01 kg) than other genetic groups. Highest body weight at 1st service and 1st calving was found to be in $1/2$ HF + $1/2$ S. The lowest age at 1st service and age at 1st calving

was observed in $1/2$ HF + $1/2$ S genetic group. Therefore, this genetic group was best economically than others. The shortest length of service period and dry period was in $1/2$ Jersey + $3/8$ S and $1/2$ HF + $1/2$ S genetic groups of animals respectively. The lactation milk yield was highest in case of $1/2$ HF + $1/2$ S genetic group in 1st. and IInd lactation and lowest in $1/2$ J + $1/8$ HF + $3/8$ S genetic group.

The value of coefficient of correlation for all the traits were found to be non significant except body weight at 1st. service, 1st. calving and length of gestation period. Milk productivity was generally associated and significantly correlated with body weight at 1st. service and 1st calving and length of gestation period. Therefore, the superiority of genetic group can be predicted mainly on the basis of these three traits.

5. Effect of Re-Breeding on Reproductive Performance in Barbari goats

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Effect of the re-breeding on estrus incidence and conception rate was studied in Barbari goats. Lactating goats were detected in heat during morning and evening hours with the help of teaser bucks after 30 days post-partum. Irrespective of stage of heat nannies were given natural service twice in same estrus and breeding was continued for a period of 40 days.

Estrus incidence was 93.1%. Incidence

of short estrous cycle was 20 per cent. Conception rate have been found to be 89.34 per cent. Fertility on the basis of nannies bred and available was 79.22 and 77.21 per cent respectively. Insemination per conception was 1.77. Twin and Triplet births were 57 and 5 per cent respectively. Reproductive performance in lactation goat was similar to that of dry goats.

6. Immunological studies of bovine semen in relation to fertility

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Semen from 4 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, against semen antigen.

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 the higher titre value obtained in the present investigation. In the serum the antibody titres were lower than the cervical mucus.

It was observed that out of 37 (71.15%) reactors 10 (27.03%) conceived, where as in 15 non reactors, 10 (66.67%) conceived.

7. Studies on the effect of Environmental temperature humidity on age at first conception in exotic cattle.

S.V. VADNERE AND S.N. LUKTUKU

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Studies were conducted on 2697 observations of calving intervals to find out the effect of lactation number (sequence of calving) on the calving interval in exotic cow breeds of Holstein Friesian, Red Dane & Jersey and their various crosses with Sindhi in Northern and Southern regions of the country.

The lactation number had a highly significant ($P < 0.01$) effect on the calving interval in Holstein Friesian crosses in Southern region. The calving interval

decreased with increase in the lactation number from 2nd to 9th lactation except in the 8th lactation in which it increased. In Holstein Friesian crosses in northern region, a decrease in the calving interval was noted from 2nd to 4th lactation only. In Holstein Friesian, Red Dane, Jersey and their crosses the effect of lactation number was not significant on calving interval.

The causes of the effect of lactation number on calving interval are discussed.

8. Influence of body weight and milk yield on the onset of post partum oestrus in Murrah she buffaloes

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Effect of body weight during 30 to 60 days postpartum on the occurrence of oestrus in 53 murrah she buffaloes was studied. 39 buffaloes showed increased body weight while 14 recorded no increase in body weight during the postpartum period extending from 30 to 60 days. Of the buffaloes that showed increase in body weight 11, 9 and 3 animals and of those with no increase in the body weight 1, 3, and 4 animals came to heat at 60, 90 and 120 days

post partum respectively. Fifty one percent of the buffaloes with the increase in body weight came to oestrus before 90 days in contrast to only 28 percent of the buffaloes that did not show increase in body weight. On the whole 58.9 percent came to oestrus by the end of 120 days when there was increase in body weight by 60 days. On the contrary only 57 percent buffaloes that did not show increase in body weight came to oestrus.

9. Studies on the Effect of Sexual Health Control in Improving Fertility in cow.

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The study was conducted on 50 non-descript village cows aged between 4 years and 6 years having the history of post-partum anaestrosity to observe the effect of three separate lines of treatment in reference to sexual health control on the improvement of their fertility. In the first line of treatment comprising deworming, improved managerial practices, correction of ration and massage of genitalia including ovaries, 14 out of 50 cows exhibited post-partum estrum and 10 cows conceived. In the second line of treatment comprising parenteral administration of Vitamin 'A' in addition to the

first line of treatment, 18 out of 36 cows which did not respond to the first line of treatment came to heat and 10 ultimately became pregnant. In the third line of treatment involving parenteral administration of synthetic estrogen in addition to the first line of treatment 12 out of 18 cows which failed to respond to the second line of treatment manifested heat and 6 cows became pregnant. The result of the present study clearly indicated that by advocating the sexual health programme, the fertility in cow can be improved to a considerable extent.

10. Studies on Certain Factors Influencing Conception rate in cows

JASWANT SINGH, B.K. SINGH

L.D.O., Artificial Breeding Centre, JAMMU

Low conception rate in cattle through artificial insemination has attracted the attention of research workers from time to time. Among many factors nervousness in females at the time of artificial insemination causes liberation of epinephrine which affects sperm transport in the genital tract. Subclinical infection present in the genital tract also in females causes early embryonic death thus reducing the conception rate. The present experiment was conducted to control the disadvantages caused due to nervousness and subclinical infection that might be present at the time of insemination.

In the present experiment, 103 cows and 45 heifers were treated with ampicillin (25 mg,) 500 mg, intrauterine one hour before insemination in different groups; Oxytocin (301.4; 501.4; I/m five minutes before insemination in different groups; Clitoral massage (for 10

seconds five minutes before insemination); Udder massage (for 2 mt five minutes before insemination.)

Normal deviate test showed significant rise of conception rate in cows in groups of ampicillin (500 mg); Oxytocin (501.4) and Udder massage as to be 77.78, 83.33% and 80% respectively compared to control percentage of 44.44 whereas all other treatments gave non-significant results except clitoral massage in combined category where C.R. was found to be 80% compared to 44% of control.

Since ampicillin, oxytocin administration, clitoral massage and udder massage were highly effective in raising the conception rates, it can be inferred that loss of conception due to subclinical infection was of similar magnitude as the loss of conception due to epinephrine release.

11. Comparative study of conception rate with chilled semen versus frozen semen under intensive cattle development project Jalna in Maharashtra

M.J. DESHMUKH

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A project for comparative study of calculating the conception rate with chilled semen versus frozen semen under Intensive Cattle Development Project, Jalna (M.S.) was undertaken since the I.C.D. Project Jalna had switched on to

the Frozen Semen Technology from Chilled Semen with effect from 1982.

A need was felt to take up this work and it is to mention that the conception rate with frozen semen was found better.

In this study considering all the

conditions were same location, period, cows inseminated, inseminator etc. The difference was taken in account with semen quality due to different preservation methods, even breeds were not considered.

The A.I. required per conception in frozen semen were also calculated and it was found much better with frozen semen than chilled semen.

The above studies also revealed that improved conception rate with frozen semen may be due to the advantage of "better preservation and non-deterioration of quality of semen during transport and storage.

Further studies covered sex ratio i.e. male and female on calves born basis in chilled semen and frozen semen insemination technology.

SESSION: III

Pathology of Parturition and Genital abnormalities

1. Prevention of retained placenta in Dairy cattle.

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An experiment was designed to study the effect of Vit. A, Selenium Vit. E, and Prostaglandin F_2 alpha on the prevention of retained placenta in dairy cattle. A number of Friesian cows were allocated to three groups (G). G.I: consisted of 100 cows, 50 of them received 2 million units of Vit. A each I/M a day before the expected date of parturition and the other 50 were served as control. G II: comprised of 48 cows, 24 cows were given a single I/M injection of Selevitan (Selenium, Vit. E) each, 21-28 days before the expected date of parturition and the other 24 left as control. G III: consisted of 45 cows, of which 25 cows were each treated with a single I/M injection of 15 mg of Luprostinol (PGF_2 alpha) 2 hours after parturition and the other 20 were left as control.

Placenta was considered normal if expelled within 12 hours after parturition, delayed if expelled after 12 hours and

retained if required interference.

The incidence of normal was 28% in the control, 68% in the treated, and that of delayed was 16% in the control and 24% in the treated and of retained 58% in the control and 24% in the treated G.I. Cows. In the G II. cows the incidence of the normal was 41.7% in the control 70.8% in the treated, the delayed was 29.2% in the control and 0.0% in the treated and the retained was 29.2% in the control and 29.2% in the treated. While in G III. cows the normal was 25% in the control and 96% in the treated, the delayed was 25% in the control and 0.0% in the treated and the retained was 50% in the control and 4% in the treated.

In conclusion, PGF_2 alpha was superior to Vit A in preventing retention of placenta, while Selenium vit. E was ineffective. We advise injection of a dose of Prostaglandin after parturition to prevent ROP.

2. Studies on some factors associated with retained Foetal membranes and its effect on Fertility of cows.

R.K. PANDIT, S.P. SHUKLA

Mhow Veterinary College

The overall incidence of retention of placenta in Gir cows and their crosses was 8.86% out of 1,117 calvings studied during 7 years. The incidence rose significantly from 5.07% in primiparous cows to 17.77% in pluripara. Seasonal differences were significant, being highest in Gir cows during spring and in $\frac{1}{2}$ F $\frac{1}{2}$ G and $\frac{1}{2}$ J $\frac{1}{2}$ G cows during summer. The number of subsequent A.I. for conception

after retention of placenta were more by 1.26 to 2.6 in different groups. Service period following retention of placenta increased by 25.6 to 78.9 days. Gestation length was less by 2.88 to 16.95 days when there was retention of placenta. The cows which became infertile or sterile subsequent to retention of placenta were: 58% in Gir, 23.53% in $\frac{1}{2}$ J $\frac{1}{2}$ G and 16% in $\frac{1}{2}$ F $\frac{1}{2}$ G.

3. Incidence and causes of abortion in Surti buffaloes

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The overall incidence of abortion in Surti buffaloes was 2.21%, 2.80%, 1.82%, and 1.12% during the years 1975-76, 1976-77, 1977-78 and 1978-79 respectively. Season had significant effect on the occurrence of abortion. During the year highest incidence of abortion cases were recorded during summer season (March to June) and lowest during winter season (November to February).

During the study 32 paired sera samples (one on the day of abortion, second sample on 21st day of abortion) were collected from aborted buffaloes at different stage of abortion. Out of 32

paired sera samples 24 were positive for Infectious Bovine rhinotrachitis (IBR) virus and four sera samples were positive for both IBR and Blue tongue virus. All the sera samples were negative for antibodies against Brucella, Chlamydia, and *Leisteria monocytogens* organisms. Serological testing however revealed more than one viral entity in causing buffalo abortion. (Diagnostic centre, N.D.D.B.)

Out of 12 foetuses examined two were positive for *Aspergillus* and *Mucor* species both, one for *Pseudomonas* spp, on cultural isolation.

4. Post abortional sexual behaviour in Surti Buffaloes

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In the present study it was observed that under the farm condition highest incidence of abortion was recorded at three to five months stage of pregnancy where as under the field condition mostly early abortions (2 to 3 months) were observed.

In 32 cases of abortions studied under the field condition, it was observed that buffaloes exhibited heat symptoms on the day of abortion in early gestation (2 to 3 months stage). Retention of foetal membranes did not occur in early abor-

tions (1 to 3 months). In very early abortions foetuses along with intact foetal membranes were usually expelled. Retention of foetal membranes was always observed when abortion took place in later half of the pregnancy. Usually the foetus was expelled with ruptured foetal membranes.

In 12 aborted foetuses, 7 (58.30%) were male and 5 (41.70%) were female foetuses. Aborted female foetuses were carried longer than aborted male foetuses (189 ♀ vs 94 ♂ days).

5. Incidence of certain types of oestrous and Pathological Termination of pregnancy in Bovines

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Data recorded in the experimental herd of the Indian Veterinary Research Institute, Izatnagar during the year 1961-65 on 274 oestruses in heifers, 1297 in cows and 334 in buffalo cows and 61 pregnancies in heifers, 359 in cows and 90 in buffalo cows were analysed. It was observed that 90.41%, 81.41% and 90.70% of oestruses in heifers, cows and buffalo cows respectively were fit for insemination. This also included induced oestrouses shown by 3.65% heifers, 7.78% cows and 10.77% buffalo cows respectively in the herd. The remaining oestruses included gestational oestrus 3.23%, 6.23% and 5.98%, oestrus with pathological genital mucus discharge

5.82%, 7.32% and 2.02% in the respective category of animals and early post-partum oestrus, occurring within 35 days following parturition, 4.54% in cows and 1.50% in buffaloes.

The observations revealed that 91.80%, 84.95% and 83.35% of the pregnancies heifers, cows and buffalo cows respectively terminated normally while early embryonal mortality was recorded in 4.92%, 7.23% and 5.55% and abortion in 1.64%, 2.54% and 4.44% in the respective group. Dystokia was observed in 1.95% cows and 4.44% buffaloes and stillbirths in 1.11% buffaloes. Animals sold during gestation were 1.64% heifers, 2.22% cows and 1.11% buffalo cows.

6. Factors associated with Dystocia in cross bred cattle, A study on 112 cases.

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A total of 112 cases of dystocia (60 cows and 52 heifers) were attended during the last two and half years in and around Ranchi. In cows, maximum incidence (83.3%) was recorded during 2nd to 4th gestation and presentational and postural defects lead to more cases of dystocia (53.3%) as compared to primipara (30.0%). In primiparous animals the major problem causing dystocia was incomplete relaxation of birth canal (59.61%). Out of 112 cases, presenta-

tional and postural defects were detected in 59 cases, out of which 83.0% were in anterior presentation. Deviation of head was the major postural defect in anterior presentation (47.4%) followed by flexion of fore limbs (33.3%). Male calves were found to be associated with dystocia more often (61.1%) than female calves (38.9%). Furthermore, the mortality rate of male calves during dystocia was considerably higher (48.5%) as compared to female calves (21.4%).

7. The Clinical Outcome of Different Regimes of Treatment of Uterine Torsion in Buffaloes

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Seventy seven pregnant buffaloes having uterine torsion were subjected either to rolling on their back or to cesarean section for detorsion and delivery of the fetus. The fresh and apparently healthy cases were rolled while the delayed and complicated cases and few cases where delivery failed following rolling, were operated for cesarean section. Uterus in 80% of the rolled animals

could be successfully detorted. The animals having fully dilated cervix at detorsion had maximum survival rate (94.7%). The survival rate was minimum where cesarean followed unsuccessful rolling. Rolling of dam has been recommended as the routine treatment of choice and cesarean section should be undertaken when absolutely necessary.

8. Biochemical changes in serum constituents of Cervico-Vaginal prolapse in Cattle

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An investigation was carried out to envisage the changes in serum calcium, inorganic phosphorus, magnesium and total protein concentration in cows having Cervico-Vaginal prolapse.

The serum calcium and magnesium levels (mg %) were 9.47 ± 0.31 and 2.20 ± 0.19 in non pregnant lactational; 9.20 ± 0.26 and 2.35 ± 0.14 in pregnant, and 7.56 ± 0.31 and 2.05 ± 0.24 in post partum cows. The level of inorganic phosphorus were found to be 5.86 ± 0.37 mg.% in non pregnant lactational, 5.70 ± 0.28 mg.% in pregnant and 5.55 ± 0.42 mg.% in post partum cows. The serum calcium and magnesium levels were significantly low ($P < 0.05$) with an increase in the level of inorganic phosphorus in all groups of prolapsed cows in comparison to

controls. The total protein (gm%) in prolapsed cows were 6.94 ± 0.34 during non pregnancy lactational, 7.69 ± 0.37 , during pregnancy and 7.29 ± 0.33 during post partum period. Though there is elevated value of protein in cows other than non pregnant lactational, the difference was not significant.

The post treatment values of all the prolapsed cows revealed a significant increase ($P < 0.05$) in serum calcium level in all groups. The calcium phosphorus ratio and magnesium value showed an increase ($P < 0.05$) in pregnant and post partum group of cows following clinical recovery. The difference in the post treatment values of inorganic phosphorus and total protein was not statistically significant.

9. Traumatic Lumbosacral and iliosacral subluxation in Parturient Buffaloes.

R.K. PATIL

Dept. of Animal Reproduction, P.K.V., AKOLA

Acute sub-luxation of lumbo-sacral and sacro-iliac joints without paraplegia is reported in three parturient Bhadwari buffaloes. Symptoms like sinking of lumbar region, sliding in of its posterior portion under the dislocated sacrum alongwith loose wrinkled skin at the area, which appeared within an hour of parturitions during straineous transport by road, gave an appearance of reduced length of the trunk and peculiar arching of the back. Except dragging movement

of the hind quarters, locomotion remained unaffected. All animals started showing steady recovery with normal progressive increase in milk yield and appreciable recovery could be observed within three weeks in the two followed-up cases. Relaxation of pelvic suspension due to hormonal changes consequent to approaching parturition, stress of transport and straining during parturition are possible attributing factors for this condition.

10. An immunological approach to assess the early embryonic losses in dairy animals.

S.K. GUPTA, R.L. DHOBLE

S.K. Gupta, R.L. Dhoble., I.V.R.I., IZATNAGAR

The failure of animal to return to estrus, following natural service/artificial insemination is usually regarded to indicate pregnancy. This is commonly confirmed by examination per rectum at 90 days after service. In the non-pregnant cases, a possibility of early embryonic loss is not easily ruled out. Furthermore, no specific methods appear to be available which can help direct assessment of loss due to early embryonic loss in dairy animals.

The recognition of Early Pregnancy Factor (EPF) in rats, mice, ewe, sow, cow, mare and woman in recent years, has opened a possibility to directly assess

losses due to early embryonic death. EPF is claimed to be detected by 72 hours after service in blood of the female. It is regarded to be secreted by ovary and fallopian tube and observed in blood of pregnant animals during first half to two thirds of gestation period. The method for detection of EPF may therefore help differentiation of animals with zygote from those without it and thus assist in estimation of early embryonic loss.

The various requirements for the detection of EPF in dairy animals and test method is discussed with its limitations.

11. Genital abnormalities of Goat.

N.C. SHARMA, S.K. GUPTA

Indian Veterinary research Institute, Izatnagar

959 genital organs from the abattoir were collected to observe the incidence of genital abnormalities in goats. The study revealed the abnormalities of ovaries, oviduct, uterus and cervix as 7.71, 2.18, 9.27 and 1.77 percent respectively. An

interesting finding was the occurrence of large cysticercus tenicollis cysts around the cervix and on the body of the uterus, which is an unusual site for this type of parasitic cysts.

12. Studies on 'White Heifer Disease' in west Bengal, A case report.

M.C. MAZUMDAR, B.N. DEY

Directorate of W. Bengal Vety-Services, Calcutta

A cross, between Jersey and Indigenous Heifer a four and a half years had one inch long vaginal opening with Short Vulva, distended abdomen.

During examination per rectum it was found that it had barrel shaped right uterine horn, no palpable ovaries.

Per vaginal examination it revealed presence of un-perforated thick fibrous

hymen.

Following usual Laparo-Hysterotomy revealed presence of Straw Colour Semi-Solid pus in the forty two inches affected uterine horn and un-ruptured fibrous hymen in the vaginal canal respectively clearing indicating as being "White Heifer Disease".

13. *Yersinia Enterocolitica* Associated with third Trimester Abortion in Buffaloes

A.M. DAS, V.L. PARANJPE, S. WINBLAD

Bombay Veterinary College, Parel—Bombay 12

Yersinia enterocolitica was isolated from a group of buffaloes aborted at the third trimester of gestational period. As per the investigations, the source of infection was probably the semen of the breeding buffalo bull. All aborted animal sera had high antibody titres against *Yersinia*

enterocolitica antigen which cross-reacted significantly with *Brucella abortus* antigen. It was possible to eliminate the infection by intra-uterine infusion of Gentamycin sulphate (160 mg/day) for three consecutive days.

SESSION: IV

Andrology and male-Sexual behaviour

Study of semen quality Holstein Friesian bulls in controlled environment in Psychrometric Chamber.

S.K. SAXENA, N.C. SHARMA

I.V.R.I., IZATNAGAR

The study was undertaken to see the effect of high temperature on the semen quality in Holstein Friesian bulls. Six adult Holstein Friesian bulls, 4-6 years of age, which were in routine semen collection by artificial vagina were taken for this study. These bulls were kept in the psychrometric chamber for 10 days at the temperature of 18.5°C at a comfortable zone to observe the semen picture and then for 22 days at a higher temperature of 37.0°C and vapour pressure 10-12 mm mercury. The semen was collected by artificial vagina and it was evaluated

for various characters. The mean reaction time (seconds), semen volume, motility, dead percentage, abnormal percentage and sperm concentration (millions per ml) at 18.5°C was 21.33 ± 2.67 , 4.61 ± 0.19 , 4.16 ± 0.10 , 10.77 ± 0.46 , 8.11 ± 0.50 and 1201.55 ± 41.49 respectively. The corresponding values at 37.0°C were 56.61 ± 9.48 , 4.21 ± 0.21 , 2.47 ± 0.11 , 17.55 ± 0.90 , 15.88 ± 0.95 and 970 ± 32.95 respectively. The study revealed a deleterious effect on the semen quality at 37.0°C which was statistically significant.

Body weight and scrotal size in relation to Age and time of First ejaculation in pubertal Nili Ravi Buffalo bulls.

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and semen production unit, Quadirabad

In the present study we are reporting data on body growth, testicular size and the age at first ejaculation in 10 buffalo bulls of the Nili-Ravi breed. The study was carried out from 18 months of age to the time when motile sperm appeared for the first time in the ejaculate. The body weight and scrotal circumference of each animal was recorded monthly. The bulls were exposed twice a week to a teaser animal alongwith other semen

donors, for serving into an artificial vagina. Age at which the animals yielded semen was recorded. The mean body weight and scrotal circumference of the 10 animals at the age of 18 months were 235.10 ± 32.60 kg and 17.12 ± 1.31 cm, respectively. The mean age at first ejaculation was 29.33 ± 1.65 m (range 28 to 33 m) of age and the mean body weight and scrotal circumference recorded at the time of first ejaculation were 449.44

± 34.50 kg and 24.04 ± 1.29 cm, respectively. Highly significant correlations were observed between age and body weight, body weight and scrotal circumference and age and scrotal circumference. One of the bulls, however, did not

ejaculate until even at the age of 37 m. It is hoped that the preliminary data obtained, would be useful in constituting suitable criteria for predicting the reproductive performance of buffalo bulls at an early age.

3. Influence of age body weight, body size on Scrotal Circumference, testis length, width, thickness of the Murrah Buffalo Males During the Post Natal Development.

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Department of Obstetrics and Gynaecology, Madras Vety.
College, Madras 7.

The mean values of the above measurements for different age groups at three months interval for 155 Murrah male buffaloes from birth to 36 months in Tamil Nadu were furnished. Their ranges from birth to 36 months were as follows: Scrotal circumference 8.7 ± 0.18 to 23 ± 1.06 cm; Testis length 3.3 ± 0.55 to 9.0 ± 0.44 cm; Testis width 2.7 ± 0.88 to 8.1 ± 0.35 cm; Testis thickness 1.5 ± 0.05 to 4.6 ± 0.76 cm; Body weight 44 ± 2.52 to 323 ± 13.84 kg; Girth size 83 ± 1.49 to 1.67 ± 2.56 cm. The testicular measurements showed an uniform increase throughout the postnatal period.

Body weight and body size were observed to be significantly correlated with scrotal circumference during 6 to 24

months of age. Tests length, width and thickness were also significantly correlated with bodyweight and body size at most of the postnatal periods. The girth size was more highly significantly and positively correlated with above parameters than body weight. Significant influence of age was seen around 6th to 12th month only. So fast growth can be expected in those periods if animals are placed in high plane of nutrition and are well cared and managed. The testis growth was first linear. Then it was antero posterior closely followed by medio lateral growth. The increase in scrotal circumference was contributed by the increase in testicular width or thickness or both.

4. Protein polymorphism and isozyme pattern of buffalo (*Bos bubalis*) seminal plasma of various Fertility.

K.C. CHAUDHARY, P.C. GANGWAR

Punjab Agricultural University, Ludhiana, 141004

Six adult healthy Murrah buffalo bulls of known fertility maintained on normal ration at the University Dairy Farm were used. Semen was collected by using an artificial vagina. Seminal plasma was obtained by centrifuging the semen at 2300 g for 20 min. The seminal plasma thus obtained was subjected to polyacrylamide gel electrophoresis at a constant current. The Protein fractions were visualized by staining the gels with 1% dye and the *rf* values were calculated with reference to marker dye. Excess of the stain was removed by diffusion in 7% acetic acid. The enzymes, alkaline phosphatase, acid phosphatase, estrase and

amylase were visualized by incubating the gels with their respective substrates.

7-12 protein components were observed. Protein components varied among the bulls. However, components at No. 3, 5, 7 and 9 were invariably present in all the cases. But the components at No. 2 and 8 were missing in the bull of low fertility. Alkaline phosphatase, Acid phosphatase, estrase and amylase showed three, two, two and a single enzyme band, respectively. The results indicate the implication of components at No. 2 and 8 in the determination of the fertility of the bull. Work on their isolation, purification and characterization is warranted.

5. Immunological cross reactivity and species specificity of seminal plasma proteins of the Indian Buffalo and cattle.

B.A. KULKARNI

Bombay Veterinary College Parel—Bombay, 12

Immunological cross reactivity and species specificity of seminal plasma proteins of the Indian buffalo and cattle were investigated by using rabbit antibuffalo seminal plasma serum, rabbit anticattle seminal plasma serum (unabsorbed and absorbed) gel diffusion and immunoelectrophoretic analysis. Gel diffusion analysis of the buffalo seminal plasma using rabbit anticattle seminal plasma serum and cattle seminal plasma

using rabbit antibuffalo seminal plasma serum indicated antigenic similarities between some seminal plasma proteins of these two species, while distinct antigenic differences were observed in their other seminal plasma proteins. IEA studies of the buffalo seminal plasma, using rabbit anticattle seminal plasma serum has revealed that out of at least 10 to 12 seminal plasma proteins of the buffalo two major and 3 to 4 minor

proteins migrating towards the anode and showing strong cross reactivity were antigenically similar to cattle seminal plasma proteins and the remaining were antigenically different and species specific for the buffalo. IEA of cattle seminal plasma using rabbit antibuffalo seminal plasma serum has revealed that out of at least 7 to 10 seminal plasma proteins of cattle, one major and 3 to 4 minor proteins migrating towards the anode

and showing strong cross reactivity were antigenically similar to the buffalo seminal plasma proteins and the remaining were antigenically different and species specific for cattle. It is suggested that the structural differences in seminal plasma proteins of the buffalo and cattle are probably implicated in physiological differences in various reproductive processes of these two species

6. Immunological studies on seminal plasma proteins of the Indian Buffaloe and cattle.

B.A. KULKARNI

Bombay Veterinary College, Parel—Bombay 12

Seminal plasma Proteins of the Indian buffalo and cattle were immunologically investigated using rabbit antibuffalo seminal plasma serum, rabbit anticattle seminal plasma serum (unabsorbed and absorbed) gel diffusion and immuno-electrophoretic analysis. At least 9 to 12 and 7 to 10 different Proteins were present in the seminal plasma of the buffalo and cattle respectively. Albumin and IgG were identified in both the species. At least 4 to 6 seminal plasma proteins of the buffalo and cattle were

antigenically similar to their blood serum Proteins. Using absorbed rabbit antibuffalo seminal plasma serum and rabbit anticattle seminal plasma serum at least 6 to 7 and 5 to 6 seminal plasma specific Proteins were observed in the buffalo and cattle seminal plasma respectively. Antigenically these Proteins were different from the blood serum Proteins of these two species. The origin and biological significance of seminal plasma Proteins are discussed.

7. Certain Characteristic Features of Epididymal Spermatozoa of Black Bengal Buck

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Luminal content of three different regions of epididymis namely (a) caput, (b) corpus, (c) cauda and (d) vas deferens was collected from 45 Black Bengal bucks by open method of castration. The parameters studied were (a) Livability of sperm, (b) Location of cytoplasmic droplet in the sperm, (c) Susceptibility of sperm to cold shock, (d) Mensuration characters of sperm, (e) Succinic dehydrogenase activity of sperm, (f) Melanizing activities of midpiece of sperm and their interrelationship in the above three regions of epididymis.

Maximum number of live spermatozoa was found in cauda part of epididymis. The number of spermatozoa showing

cytoplasmic droplets, resistance to cold shock and the lengths of midpiece of spermatozoa gradually decreased while both succinic dehydrogenase and melanizing activities increased progressively during the transit of spermatozoa from the caput to vas deferens i.e. during the maturation process of spermatozoa.

It might be concluded that the presence of cytoplasmic droplet, susceptibility to cold shock and the length and the area of midpiece of spermatozoa were found to be inversely related with percentage of live spermatozoa, succinic dehydrogenase activities of spermatozoa during the process of maturation of spermatozoa of Black Bengal buck.

8. Studies on the Effect of Glass Wool Column Filtration on the Quality of Semen in Bovines

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Madras Veterinary College, Vepery—Madras 600007

Semen from normal healthy cross bred bulls and from bulls with andrological problems were subjected to glass wool column filtration. Based on initial spermatozoal motility and viability they were categorised as good or poor quality semen. Disposable pasture pipettes of 12 cm. length and with an inner diameter of 0.75 cm. were loosely but evenly packed with 40-50 mg. of borosilicate glass wool fibres, to a length of 2 cm. Semen was allowed to filter through the glass wool column by gravity.

Glass wool column filtration resulted in significant increase in spermatozoal motility and decrease in spermatozoal concentration and percentage of dead

and abnormal spermatozoa. Cold shock index and preservability at 5°C and freezability at -196°C were increased significantly after filtration. All these beneficial effects of filtration were more marked with poor than with good quality semen. As the levels of dead and abnormal spermatozoa increased in the semen sample their retention in the column also increased. Sperms with tail abnormalities were retained more effectively than those with head abnormalities. Semen filtrate contained more live and morphologically normal spermatozoa than the original semen sample emphasising the improvement in the quality of semen after glass wool column filtration.

9. Sloughing cell Filtration: A Major Function of Caput Epididymis in Goats.

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Fifty eight pairs of testes and epididymis of 1-2 year old goat were collected on slaughter. Histology, histoenzymology, pH of testes and epididymis were done. Fluid from testes and different parts of epididymis were collected and Na and K ions and free amino acid concentration was measured. The total plasma; cell ratio was worked out of this fluid.

It was noted that sloughing cells were present into the lumen of seminiferous tubules, rete testes and caput, however, these cells were not present in corpus and cauda. The pH was found to be slightly alkaline at the testes which became very high at caput and slowly comes to normal or slightly acidic at cauda. The alkaline phosphatase activity

was very less in testes and very high at caput which decreased in corpus and cauda. The acidic phosphatase activity is less in testes and caput but was maximum in corpus followed by caput.

The Na and K ions concentration was maximum in caput followed by cauda and testes. The free amino acids concentrations was more than two fold in caput in comparison to cauda fluid. The plasma: cell ratio was maximum in caput followed by testes and cauda.

It was informed that the sloughing cells were being eliminated by the caput epididymis by dissolving these cells, thus filters the mature spermatozoa for further maturation in corpus and cauda.

10. Seminal fructose and reaction time in cross bred bulls.

K.V. PATEL, A.J. DHAMI, V.M. MEHTA S.B. KODAGALI

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A study on "Andrological investigation of nine cross-bred bulls" was undertaken during the period of one year (cold, hot and wet seasons) at the Department of Veterinary Obstetrics and Gynaecology Gujarat Veterinary College, Anand. The study included clinical examination, evaluation of seminal characters, assay of seminal biochemical constituents and estimation of haematological parameters. In this study a total of 192 ejaculates were collected and reaction times were noted. Reaction time was considered as the time interval (in seconds) between the period bull was in proximity of the

teaser till donation of semen. Initial fructose content was assayed in neat semen samples according to Mann (1948).

The mean reaction time and fructose content in semen samples of nine cross-bred bulls were compared for the study. It was found that as the reaction time increased, there was reduction/decline in the level of initial fructose content in semen samples. This indicated the existence of a negative correlation between these two characters. Seminal fructose levels indirectly communicated the levels of testosterone in the bulls under study.

11. Harmonal Treatment of Lack of Libido in Murrah Buffalo Bulls

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Madras Veterinary College Madras 600007

Lack of libido in young Murrah buffaloes is commonly met as a field problem in Tamilnadu. In the report four progeny tested Murrah buffalo bulls of $2\frac{1}{2}$ years of age of an organised farm, suffering from lack of libido was treated with lutinising

hormone 4500 IU intramuscular every third day for three times. All the treated bulls showed excellent libido within 10 days of treatment. The two untreated control animals resumed libido well after 120 days.

12. The optimum time Frequency of sperm output for native Ram

A.K. FAZLUL HAQUE BHUIYAN, M.A. HASNATH

Bangladesh Agricultural University, Bangladesh

Semen was collected from two native breeding rams for six different frequencies to see the effect of ejaculation frequency on the semen characteristics for use in A.I. programmes. The ejaculation frequencies varied from one to six times per week. The results thus obtained from the experiment has indicated no significant differences in semen volume (ml.) motility (%), and live count (%) of spermatozoa at different ejaculation frequencies; but the trend showed that mean semen

volume was highest at the ejaculation frequency of once in a week. Significant differences ($P < 0.05$) in abnormality (%) and concentration (millions/ml) of spermatozoa at different ejaculation frequencies were observed. It may, therefore, be inferred from the experiment that 2 to 3 times collection frequency can be followed in the schedule of semen collection from native ram without deteriorating the quality of semen.

13. Clinical Feminisation in a Bullock.

S.B. KODAGALI

Gujarat Veterinary College, Anand—388001

A clinical feminisation condition was met in a bullock with enlarged teats and hypertrophy of mammary tissue. On detailed examinations and the history of the animal, Sertoli Cell Tumours in retained testis with metastasising condition could be diagnosed. Endocrinologi-

cally, the tumours were active as oestrogens produced by sertoli cell tumours with retained testis resulted into the observed clinical feminisation condition. The work efficiency of the animal was not markedly affected but the unsightly appearance.

14. Effect of Prostaglandin F₂ Alpha on Libido Seminal Quantity and Quality of Buffalo Bulls

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J. RAMAMOHANA RAO

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Four mature Murrah buffalo bulls on a regular semen collection schedule of 2× on each of 2 days per week were injected 30 mg prostaglandin F₂ alpha THAM salt intramuscularly 30 minutes prior to each alternate first ejaculation for 6 weeks. Effects of PGF on time to initial false mount on the decoy, time to ejaculation after two false mounts (reaction time), seminal volume, sperm concentration, total sperm output, motility of fresh and thawed semen and the semen doses obtained per collection were evalua-

ted. Treatment caused significant ($P < 0.01$) reduction in the time to first false mount and the reaction time for the first ejaculations but had no effect ($P < 0.05$) on these for the second ejaculations. Values for other quantitative and qualitative parameters did not differ ($P < 0.05$) due to PGF treatment. It is concluded that PGF at the dosage and frequency of administration used may be of some value in improving libido in low libido bulls but does not alter the reproductive capacity of buffalo bulls.

SESSION: V

Female Reproductive Performance

1. Effect of Body condition at calving on subsequent fertility in buffaloes

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The data on 180 normal calving buffaloes were used to study the effect of body-condition at calving on postpartum reproduction performance. A technique for scoring body-condition of animals developed at East of Scotland, College of Agriculture (ESCA) was used, which is simple and easily learnt. The buffaloes were awarded an appropriate body-condition score at calving on the Scale 1 (Very thin) to 5 (very fat) and later classified as Thin, (score upto 2), Medium ($2\frac{1}{2}$ to $3\frac{1}{2}$) and Fat (4 and above). Maximum (91.7%) conception was observed in buffaloes with body-condition score $3\frac{1}{2}$, which was considered as 'Target condition score' at calving. The overall conception

rate was significantly ($P < 0.05$) higher (88.3%) among buffaloes with medium body condition as compared to thin (65.8%) and fat (70.8%) buffaloes. The first postpartum estrus period of medium and fat buffaloes was significantly ($P < 0.01$) lower (66.2 and 66.9 days) than the thin buffaloes (77.7 days). The service period of buffaloes with medium body-condition at calving was also significantly ($P < 0.01$) shorter (128.3 days) than fat 144.1 days) and thin buffaloes (164.5 days). To get best postpartum reproductive performance, the buffaloes should be managed and fed in such a way so that they are neither thin nor very fat at the time of calving.

2. Studies on oestrus incidence in local cattle under rural management practices

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Asstt. Director of Animal Husbandary Nasik

Record of 34572 inseminations in 25929 local cows, belonging to cultivators, maintained at 56 units under five RAICs of ICDP Jalna, for the period of 1973-1981, was studied for knowing the incidence of oestrus in local cows under rural management. There were highly significant differences between months,

seasons and regions in respect of incidence of oestrus. Breeding activity was highest during October and Winter Season. The lowest incidence noted in April and May. The differences attributed to stress factors namely atmospheric temperature and low nutrition.

3. Some aspects of reproductive performance of Jersey cows in Assam

B.K. BARAH, G.D. SINGH, H.C. PANT

College of Veterinary Science, & A.H. Mathura (U.P.)

Breeding data of 145 Jersey cows (from 1974 to 1983) consisting of 413 calving intervals were collected. Calving interval, calving to first oestrus interval, interoestrus interval, calving to first service interval, interservice interval, calving to conception interval and gestation length were 401.6 ± 4.19 , 88.4 ± 1.99 , 33.1 ± 2.05 , 88.9 ± 1.98 , 33.9 ± 2.29 , 123.8 ± 4.19 and 277.8 ± 0.24 days respectively. The services per cow calving averaged $1.88 \pm$

0.08. Days lost due to missed heat periods was 37.6 ± 2.92 days. These results show a near satisfactory reproductive performance of Jersey cows at Barapeta Farm. However, further improvement in the breeding efficiency can be brought about by improving the efficiency of oestrus detection and inseminating cows on or after 50 days postpartum. These procedures would achieve a 365 days calving interval.

4. Incidence of Oestrous and Conception rate in Nondescript Rural Buffaloes.

SATISH KUMAR

Indian Veterinary Research Institute
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Seasonality and breeding performance of buffaloes, the chief milk producer of our country, has been reported. Most of the available informations are on farm buffaloes. An attempt was made to study the incidence of Oestrus and its relation to conception under various factors in the nondescript rural buffaloes. In about 80% of the buffaloes the onset of heat occurred during cooler hours of morning (31.30%) and evening, (48.78%). Maximum number of buffaloes (60.95%) and buffalo heifers (58.56%) were observed

in heat from November to February, while minimum number of animals came in heat from May to August. Maximum number of buffaloes (46.28%) and heifers (46.74%) were presented in mid heat for insemination. Conception rate was maximum for the buffaloes (59.00%) and heifers (57.14%) inseminated during the late heat (15 to 20 hrs.). There was no difference in the conception rate of buffaloes and heifers inseminated in first or subsequent heats.

5. Sexual maturity in crossbred heifers (zebu \times H.F.) in relation to age, body weight and uterine size.

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Southern Regional Centre, N.D.R.I. Adugodi, Bangalore

The average age at maturity (first detectable oestrous) in the Zebu Holstein (75% inheritance) was 679 days at 217 kg ($n=32$) body weight. The average per day increase before maturity, between I & II and II & III oestrous cycles were 228 gm, 529 gm and 338 gm respectively. There were significant differences in per day body weight gain between pre and postpubertal periods indicating the anabolic effects of the steroid hormones.

Similarly there were positive correlations between age & body weight and uterine size & body weight. The average interval between I & II, and II & III oestrous cycles were 68 and 33 days respectively. The significant changes is the body weight between pre and postpubertal periods and the shortening in the cycle days between the first and the subsequent oestrous cycles indicate a stabilization process in the entire reproductive systems.

6. Studies on Gestational oestrus in Surti Buffaloes

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Gujarat Veterinary College, ANAND

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 AI Clinic, Anand. There were 45 (0.64%) gestational oestruses among 42 (5.12%) pregnant buffaloes 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/AI 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%, with holding 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 in 57.78% vs 68.76% and restlessness and excitement 46.47% vs 56.17%, the differences being non-significant ($P<0.05$). 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 highest (28.57%) in animals of 3rd lactation followed by 2nd (23.81%), 1st and 4th (14.29% each). The occurrence was found to be low in heifers (9.52%) and in animals above 5th lactation. Among buffaloes exhibiting gestational oestrus, pregnancy was 44.80% in left horn and 55.20% in the right horn, the difference being non-significant.

7. Seasonal variations in postpartum ovarian activity and Fertility in Buffaloes.

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Postpartum ovarian activity and fertility were evaluated using 12 Murrah buffaloes in each group of summer and winter calvers.

Initiation of follicular development was earlier among winter calvers (29.42 days) than the summer calvers (34.08 days). However, manifestation of first postpartum estrus was significantly ($P < 0.05$) earlier in summer (37.82 days) than in winter

(84.58 days). Buffaloes that calved in summer had shorter service period (115.00 days) but required more number of services per conception (2.40) compared to those that calved in winter (136.38 days and 1.63, respectively). The first service and overall conception rates were higher among winter calvers (36.36 and 72.72%) than summer calvers (18.18 and 45.45%).

8. Studies on estrus and estrus cycle in Red Dane, SRB and Jersey Cross Breeds.

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Department of Gynaecology & Obstetrics, Nagpur
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8, 16 and 261 estrus cases were studied in Red-Dane, SRB AND Jersey cross bred heifers/cows respectively.

Manifestation of estrus symptoms were more distinct in SRB Cross breeds. There was no difficulty in detecting the estrus in all the *Three* types of cross bred studied. Animals manifesting intense and intermediate types of estrus were found more active, alert, restless, prepared to get sniffed and mounted by other herdmates.

There was absence of these behavioural changes in some of the animals with weak type of estrus.

The average length between the two observed estrus cycles was 32.67 ± 18.50 days (Range 21 to 54 days) in Red-Dane cross bred females; 43.40 ± 20.31 days (Range 22 to 64 days) in SRB females and 32.10 ± 19.29 days (Range 18 to 120 days) in Jersey cross bred females respectively.

9. Haemoglobin polymorphism in relation to puberty & sexual maturity in Gir Heifers.

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Haemoglobin polymorphism studied by vertical paper electrophoresis showed that of the 64 heifers, 23 had type A, 24 had type AB and 17 had type B. The gene frequency of Hb *a* and Hb *b* were 0.547 and 0.453 respectively.

The age at puberty was 857.00 ± 87.58 , 835.78 ± 72.84 and 899.23 ± 102.27 days for Hb types A, AB and B respectively. This indicated superiority of heterozygotes over both homozygotes.

The average age at sexual maturity was 905.15 ± 108.76 , 877.54 ± 107.15 and 954.76 ± 103.37 days for heifers with Hb types A, AB and B respectively.

The highest coefficient of correlation between the age at puberty and sexual maturity was observed in heifers with Hb type AB ($r = +0.9657$).

No other haemoglobin variant was observed. The ratio of heterozygotes to the homozygotes was 1.5:1.

10. Studies on the serum progesterone concentration of Gir cows during different phases of oestrous cycle.

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Blood serum of seven normal cycling cows of Gir breed maintained at Cattle Breeding Farm Kandivali, Bombay were collected on the day of oestrus and on every alternate day thereafter i.e. 2nd, 4th & 6th day etc. till they expressed subsequent oestrus. Blood progesterone was estimated by RIA technique.

Mean serum progesterone levels of these cows on the day of oestrus were found to be 0.28 ± 0.32 ng/ml. The serum progesterone levels (ng/ml) on 2nd, 4th, 6th, 8th, 10th, 12th, 14th, 18th and 20th day were found to be 0.31 ± 0.033 ; 0.52 ± 0.14 ; 1.76 ± 0.26 ; 3.29 ± 0.20 ; $4.70 \pm$

0.19 , 5.58 ± 0.15 ; 6.26 ± 0.12 ; 6.24 ± 0.25 ; 2.23 ± 0.16 and 0.42 ± 0.3 respectively. Serum progesterone levels of 3 cows which expressed subsequent heat on 22nd days was 0.30 ± 0.3 ng/ml. It was observed that the minimum progesterone level was on the day of oestrus with a steady and gradual increase in the progesterone levels upto day 12th. The serum progesterone levels were noticed to be on higher side from 12th to 16th day of oestrous cycle with a peak value on 14th day. There was sudden depletion in the serum progesterone levels from 18th day onwards.

11. Protein bound iodine levels of normal cycling animals during different phases of oestrous cycle and during post-partum anoestrous period.

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Bombay Veterinary College

Six normal cycling cows were randomly selected for the study of PBI levels during different phases of oestrous cycle. PBI levels on the day of oestrus, and on 10th day of oestrous and 18th of oestrous cycle were 7.75 ± 0.20 ug%, 6.65 ± 0.27 and 5.62 ± 0.20 ug% respectively. There was highly significant difference between phases of oestrous cycle. PBI levels

on the day of oestrous were found to be significantly higher ($P < 0.01$) than 10th day and 18th of oestrous cycle.

The mean serum PBI levels in case of 36 cows in post-partum anoestrous condition were found to be 3.16 ± 1.15 ug%. PBI levels of normal cycling animals were significantly higher than that of anoestrous cows.

12. Gross observations on the growth and development of gravid uterus during various stages of gestation in goat (*Capra hircus* L.)

M. S. KADU & A. S. KAIKINI

Punjabrao Krishi Vidyapeeth, AKOLA

In all 330 gravid caprine genitalia in various stages of gestation, collected from abattoir were studied.

The weight of the gravid uterus increased from 35.40 to 3574.75 gm, circumference from 4.35 to 43.8 cms, length of gravid cornu from 18.37 to that of non-gravid cornu from 17.47 to 8.52 cms, 55.35 cms in first to ninth stage of gestation respectively in single pregnancy. These values from second to seventh stage in twins were 117.38 to 3375.22 g. for weight 7.15 to 33.1 cms for circumference and 20.05 to 77.24 cms for length of gravid cornua. All the four parameters were significantly higher for the right gravid cornu in singles but not in twin pregnancy.

The weight of the gravid uterus increased almost 100 times where as the circumference and length of gravid cornu increased to 10 and 4.5 times respectively from first to the terminal stages of pregnancy.

Daily growth-rate of gravid uterus increased from 2.82 to 7.71 g upto fourth stage, rapidly thereafter from 22.21 in fourth to 46.21 g at the end of seventh stage and reduced subsequently to 19.41 g. in ninth stage. The empty uterus increased from 1.001 g/day in second to 19.54 g/day ninth stage in single pregnancy.

The polynomial regression curves were drawn with the prediation equation for weight the gravid uterus in early, mid and later pregnancy.

13. Some observations on reproductive performance in sheep

S.A. KOPPAR

Gujarath Sheep and wool development corporation Ltd.

Observations on reproductive performance of Large animals like cattle and buffaloes are recorded and published. But similar observations are meagre in small animals like sheep and goats.

The Department of Animal Husbandry in Gujarat State had imported 15 Russian Merino Ewes and 3 Rams from U.S.S.R. during 1965. The selective breeding under controlled farm conditions produced 93 progeny. By the year 1973-74 the entire stock proved infertile and sterile and hence by the year 1976-77, the entire stock was extinct.

The Russian Merino rams were also used for cross breeding with Patanwadi ewes, producing 2000 cross bred progeny.

Gradual decrease in fertility in both rams and ewes were noticed.

The Gujarat Sheep & Wool Development Corporation imported 100 Russian Merino and 1250 ewes from U.S.S.R. during 1971 to 1973. During the period of 10 years from 1973-1983, 1663 progeny was born. By the year 1984, there are only 160 progeny left. Low fertility and high mortality were responsible for decrease in number.

During this period 19453 local ewes were inseminated.

75 Australian and 29 Rambouillet rams were imported during 1981-83. 121 Rambouillet ewes were imported from U.S.A. The results of selective/cross breeding are discussed.

14. Reproduction in sheep S. Effect of gestation length and dam's weight at lambing and service on preweaning body weight in exotic fine wool breeds.

S.K. KAUSHISH, P.S. RAWAT and D.B. KALRA

Central Sheep and wool Research Institute, Rajasthan

Lambings of 249 Russian Merino and Rambouillet sheep were studied under semi-arid conditions. Relationship between pre-weaning body weights of lambs and dams weights at different stages of reproductive cycle and gestation length were studied. Significant ($P < 0.05$) differences were observed between breeds

in dams weights at lambing, gestation length and preweaning 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.

15. Comparative study of reproductive parameter at plains versus at an altitude-7500 feet.

MAN MAL HARSH

Indian Veterinary research Institute, Izatnagar

The comparison of the reproductive parameter of the animals reared at Izatnagar and transferred to Mukteshvar at an altitude of 7,500' showed the inter-calving period of mean 414.27 days with range 310 to 875 increased to 58.81 days with range from 294 to 1490, the gestation period 288 with range from 218 to 378 decreased to 281 with range from 246 to 383, post partum breeding interval of mean 97 with range from 22 to 440

increased to 193 with range from 21 to 1217 days, the post service anoestrous of mean 69.23 day with range from 38 to 197 increased to 122.46 with range 30 to 353, the oestrous cycle length mean 21.02 with range 14 to 26 decreased to 20.42 with range from 8 to 26 and the service per conception was 1.58 with range 1 to 9 increased to 1.85 with range 1 to 13 respectively.

16. Impact of cross breeding of Gir Cattle with Exotic Holstein Friesian and Jersey on productive and reproductive performances of cross breeds at the Cattle Breeding Farm at Kandivli and Betegaon.

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Institute for Research & Development of Dairy Cattle,
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The years assessment has been done on productive and reproductive performances of Gir; Gir×HF and Gir×Jersey at the Cattle Breeding Farms, Kandivli and Betegaon to study the various parameters such as 1) Age at puberty 2) Age at 1st. fertile heat 3) Age at first calving 4) Service period 5) Gestation period 6) Inter caving period 7) Lactational yield 8) Lactational length 9) Wet average.

The results are as under:

The above studies indicate that cross breeds Gir×HF and Gir×Jersey have proved their superiority on various parameters as compared to Gir, specially in respect of early maturity and lactational yields. The data show good promise in

undertaking cross breeding programme of Gir×HF and Gir×Jersey.

	GIR	Gir×HF	Gir×J.
Pubertal age (days)	1060	545	468
Service period (days)	226	154	102
First Fertile heat (,,)	1125	610	536
Gestation period (days)	279	272	274
Intercalving period (,,)	487	411	392
Lactational yield (kg) 1st.	1364	2026	1722
„ „ „ 2nd.	1529	2244	1909
„ „ „ 3rd.	1442	2690	2024
Lactational length (days) 1st.	342	338	315
„ „ „ 2nd.	313	299	289
„ „ „ 3rd.	293	300	289
Wet average (kg) 1st.	4.16	5.93	6.09
„ „ 2nd.	4.77	7.54	6.69
„ „ 3rd.	4.79	8.54	7.52
Total average (kg) 1st.	2.95	4.96	4.83
„ „ 2nd.	3.53	6.17	5.26
„ „ 3rd.	3.32	6.85	5.32

17. Ovarian activity and uterine involution during postpartum period in Murrah buffaloes

T.G. DEVANATHAN and SYED ABDUL QUAYAM

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Madras Veterinary College

Thirty five pluriparous and nineteen primiparous Murrah she buffaloes located in the Central Breeding Farm, Alamadhi, Tamil Nadu were utilized for the study. The ovaries of postpartum buffaloes from 5 to 50 days were examined per rectum and according to the most striking palpable changes the ovarian condition was classified as a) hard, flat and rough, b) soft smooth and globular and c) globular with palpable corpus luteum.

Ovaries were hard and flat with rough surface in all pluriparous buffaloes upto 15 days postpartum. By 22 days postpartum 22.85 percent of buffaloes had soft, smooth and globular ovaries. In primiparous animals even at 15 days postpartum 5.26 per cent of buffaloes showed smooth globular ovaries. Palpable corpora lutea were first detected by day 22 in 5.26 per cent primiparous and by 29 days in 14.29 percent of pluriparous animals. By day 43 none of the buffaloes had hard, flat and rough ovaries. They were either soft, smooth and globular or globular with palpable corpus luteum. At 50 days postpartum 80 percent of pluriparous animals showed palpable

corpus luteum in contrast to 68.43 per cent of primiparous animals. The ovaries resume cyclical activity much earlier than the external manifestation of oestrus. There seem to be no similar work reported so far comparing the primi and pluri animals. So, in the absence of relevant reports, this difference could be explained as the effect of parity age and milk yield. Feed was unlikely to be the factor as all animals were fed with the same ratio.

The diameter of gravid and non gravid horns and cervix of the involuting uterus was measured by rectal palpation during the postpartum period extending from 5 to 50 days. The size of the gravid horn was 16.13 cm at 5 days but reduced to 5.43 c.m. by 15 days registering nearly 70 per cent of involution. Similarly the non-gravid horn size was reduced from 6.44 cm to 4.13 cm indicating nearly 37 per cent involution. Primiparous buffaloes also showed similar trend of involution changes. Subsequent to 15 days there was only gradual decrease and the uterus attained non gravid size by 36 days. The involution of the cervix was gradual and uniform from 5 to 36 days.

18. Effect of uterine horn on certain Reproductive Traits in Surati Buffalo.

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The data on a total of 95 pregnancies in Surati buffalo was studied with a view to know the effect of uterine horn on the gestation length, birth weight of calf, uterine involution, service period and intercalving period. In 55.5% cases the pregnancies were carried in the left horn indicating that probably left ovary was more functional. The gestation length was not influenced by the gravid horn. The birth weight of calf was non significantly higher in the calves carried in left horn. Uterine involution was faster in left gravid horn (24.19 ± 0.91 days), than the right gravid horn (27.00 ± 0.93 days). The shifting of the cornua in two

subsequent pregnancies occurred in 61.3% cases whereas in 38.7% cases two subsequent pregnancies occurred in the same horn. When the side of pregnancy shifted the calving to conception interval was 103.23 ± 9.10 days, and in cases of non shifting it was 99.93 ± 13.20 days. When the two subsequent pregnancies occurred in left horn only, then it was found to be still lower. Alike service period the intercalving period was also found to be lower in cases of nonshifting (419.0 ± 16.63 days) than in cases of shifting of the pregnancy into contralateral horn (425.85 ± 10.90 days).

19. System for buffalo calf rearing to reduce mortality rate and achieve early maturity.

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A system of management for callf rearing was applied with 93 calves weaned at birth. Apart from normal managerial practices of new born, a specific feeding schedule was followed. Average weight gain per day was 440 gm. from birth to maturity leading to an average of 240 kg. around 450 days of age. Female calves established regular cyclicity by 14-15 months and male could give fertile service at the age of

16-17 months. Histology of the testis revealed sperm production by 13-15 months. This was corroborated by biochemical studies of testis. Blood serum analysis revealed that higher and sustained Gamma globulin levels were observed for calves fed colostrum within 15 minutes. This system could help to reduce calf mortality to 5 to 7% and better growth for early maturity in both the sexes.

20. Biometrics of genital organs in Bannur ewes and surti does

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Parel, Bombay—400 012

10 Bannur ewes and 5 Surti does of similar, age were selected for this study. The average body weights of ewes and does being 18.52 and 17.3 kg respectively. The measurements of the various components of the genital tract were undertaken immediately after slaughter. These indicated vulva, vagina, cervix, corpus uteri, uterine cornua, fallopian tubes and ovaries.

The study revealed that the normal genitalia of both species resemble that of bovines. The colour of genitalia in ewes varied from pink to pale white whereas that of does varied from pale white to white. A distinct cul-de-sac was present on the dorsal aspect in fornix. The cervix was distinctly palpable and consisted of two lip like structures. The average weight of genitalia including ovaries was 45.7 ± 9.226 g in ewes and 79.0 ± 4.46 g in does. 80% of animals revealed 4 cervical folds in ewes and 20% had 5 cervical folds; whereas all the Surti does showed presence of 4 cervical folds. 4 rows of caruncles were present in both the cornua in both the

species. The number of caruncles in right and left and in both the cornua together was 43.8 ± 3.116 and 45.8 ± 1.913 and 89.6 ± 3.113 respectively in ewes. The corresponding figures in does were 54.6 ± 3.7 and 61.6 ± 2.135 and 111.2 respectively. Only one organ in ewes revealed the presence of a vertical band starting from the roof to the floor of vagina. No such abnormality was observed in does.

The comparison between the component parts between the species revealed that weight and length of genitalia was significantly higher in does than those in ewes. The number of caruncles was highly significant in does. The length, width and the weight of the right ovary was highly significant in does. The vagina in does shows significant increase in size suggesting further studies if easier deliveries and smoother intra vaginal corrections are possible in caprine dystocia. Similarly the increase in width of uterine cornua suggests the possibility of better prenatal development of does.

21. Some observations on gravid genitalia of bannur ewes and surti does

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Department of Animal Reproduction, Bombay-Veterinary College,
Parel, Bombay—400 012

Observations on gravid genitalia of 18 Bannur ewes and 7 Surti does were recorded in respect of circumference of uterine cornua, weight of ovaries on gravid and non gravid side, placentomes in gravid and non gravid horn and total number of placentomes in both horns together. Maximum size of placentomes

were recorded at different stages of gestation. The average volume of amniotic and allantoic fluids, colour and consistency were recorded during 1st, 2nd, 3rd and 4th month of gestation in both species. Biometrical studies of these parameters are discussed.

22. Effect of gestation length and dam's weight at lambing and service on preweaning body weight in exotic fine wool breeds.

S.K. KAUSHISH, P.S. RAWAT, D.B. KALARA

Central Sheep & Wool Research Institute Avikanagar (Via: Jaipur) Rajasthan.

SESSION: VI

Hormonal levels and Biochemical profile

1. Changes in the levels of blood glucose and free fatty acids in relation to post partum estrus in Buffaloes.

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A study using 24 Murrah buffaloes was conducted to determine the changes in the levels of blood glucose and free fatty acids (FFA) in relation to postpartum estrus. The mean values of glucose and FFA in buffaloes that exhibited postpartum estrus earlier than 45 days (Group I) between 45 to 90 days (Group II) and after 90 days (Group III) were 58.96 ± 1.37 , 55.43 ± 2.15 and 51.90 ± 0.95 ; and

39.44 ± 1.57 , 31.11 ± 1.32 and 29.27 ± 1.18 mg %, respectively. The group means differed significantly ($P < 0.01$) from each other in both parameters. In all the groups glucose increased significantly ($P < 0.01$) from the day of calving onwards with different peak periods, while FFA decreased significantly ($P < 0.01$) and almost linearly from the day of calving up to day 95 postpartum.

2. Post partum changes in sex steroids and gonadotrophins profile in relation to ovarian cyclicity in Murrah Buffaloes.

I.S. LOHAN, M.N. RAZDAN, M.L. KAKER

Haryana Agricultural University, Hissar 125004

An investigation was conducted on nine Murrah buffaloes to quantify plasma sex steroids and gonadotrophins during postpartum period till resumption of ovarian activity. After calving, plasma progesterone levels showed decline and remained low (0.02 to 0.53 ng/ml) till day 39 postpartum in most of the buffaloes. Elevated progesterone levels for short durations (4 to 5 days) occurred in buffaloes which showed resumption of ovarian activity. At estrus, progesterone levels were observed to be low in all the buffaloes. Plasma estradiol-17B levels decreased markedly after parturition and fluctuated widely during the early postpartum periods (below 3.12 to 7.70 pg/ml). Estradiol-17B levels were elevated at estrus. Plasma LH levels were low

and varied with narrow fluctuations up to day 15 postpartum. Thereafter, its level showed slight increasing trend in most of the animals and elevated levels (3.98 to 20.67 ng/ml) were observed on the day of estrus. Plasma FSH levels were also low with narrow fluctuations till day 5 to day 9 postpartum. Thereafter, FSH levels showed slight increase. Elevated FSH levels were concomitant with increased LH levels at estrus.

It may be concluded from this study that in buffaloes postpartum ovarian activity was resumed later than cattle. Buffaloes showed irregular short progesterone cycles, anovulatory estrus and silent estrus before resumption of regular estrus cyclicity.

3. Prepartum and immediate post partum plasma progesteron, 17B-estradiol, LH & FSH profile of Murrah Buffaloes (*Bubalus bubalis*)

I.S. LOHAN, M.L. KAKER, M.N. RAZDAN

Haryana Agricultural University Hissar 125004

Blood sample collected daily from five Murrah buffaloes (four pluriparous, one primiparous) over a varying period of time during prepartum stage and upto two days after parturition were analysed for progesterone, estradiol-17B, LH and FSH concentration using radioimmunoassay technique. Plasma progesterone level varied from 0.50 to 3.75 ng/ml from day 79 to day 21 prepartum. Thereafter, the levels ranged between 0.62 and 1.67 ng/ml until day 3 prepartum. By 72 to 24 h before parturition the progesterone level fell considerably (0.10—0.36 ng/ml) and continued to be low

after parturition. Estradiol-17B level varied between 8.00 and 32.00 pg/ml upto day 21 prepartum. The level then increased to the maximum (60 to 120 pg/ml) on the day of parturition, thereafter there was a steep and significant fall in the plasma estradiol level. LH and FSH levels were low and fluctuated within a narrow range from day 79 prepartum till two days after parturition. The results reveal that very high level of estradiol associated with very low level of progesterone appears to play a significant role in the process of parturition in buffaloes.

4. Oestrous cycle control by means of prostaglandins and the levels of plasma and milk progesterone in dairy cattle.

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The use of prostaglandin F2 α or its analogue cloprostenol in the management of reproduction of dairy cattle has proved far more easier to obtain better fertility in heifers compared with cows. PGF2 or its analogue cloprostenol when

administared into cattle with a palpable corpus luteum is capable of inducing pre-mature regression (luteolysis) followed by oestrus and ovulation.

In a series of experiments with Friesian heifers it was observed that more than

90% of heifers show oestrus within 96 h after the second of two injections of cloprostenol given 11 d apart and 70% become pregnant to A.I. 72 h and 96 h after the second injection. The patterns of change in levels of progesterone in the plasma of these heifers resembles very closely that found at the time of natural regression of the corpus luteum and subsequent oestrus and ovulation. On the other hand, control of the timing of oestrus by means of two injections of cloprostenol given 11 d apart to Friesian cows has resulted in 40-70% showing oestrus 48-96 h after

the second injection. A.I. 72 h and 96 h after this resulted in pregnancy rates of about 35%. Many of the cows that did not so respond had milk progesterone concentrations markedly different from those characteristic of normal period of oestrus, ovulation and corpus luteum formation. The drug is equally effective in causing rapid luteolysis in heifers and cows and the failure of many cows then to show oestrus and ovulate within 3-4 days of luteolysis indicates some degree of deficiency of ovarian function at this time after calving.

5. Plasma FSH and LH concentrations after Cloprostenol Injection at pro-oestrus and Oestrus in the dairy Cow.

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Two hours after the cloprostenol injection, there was an immediate increase in plasma L.H. concentration in 1 of 5 cows (cow X430) 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 extent from one cow to another.

Four of 5 cows showed a pre-ovulatory F.S.H. surge and in 3 cows, the L.H.

surge. The time of occurrence of these peaks varied between cows. The mean time lag between the gonadotrophin surges and the Cloprostenol injection 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.

6. Oestrus Behaviour and Annual Pattern of Peripheral Progesterone in Crossbred Cows at Two Levels of Nutrition

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The present study was undertaken to elucidate whether the level of nutrition or environmental temperature or both are responsible for infertility in crossbred cows. Twelve non-lactating normal cycling crossbred cows were distributed into two groups of six each and were fed on maintenance ration in group I and on sub-maintenance ration in group II, for a period of one year. The calendar year was divided into two seasons viz., summer from April to September and winter from October to March. Throughout the period of study, average, weekly, minimum and maximum temperatures and relative humidity were recorded. Routine detection of oestrus was practised with the help of teaser bull and through behavioural symptoms. The blood samples were collected on alternate days from

all the cows by jugular venupuncture. Progesterone concentration in blood plasma was determined by radio-immunoassay technique. Average anoestrous periods in groups 1 and 2 were 36 and 170 days respectively. Out of six cows in group I, three showed normal cyclicity throughout the year, whereas all the six cows in group II showed anoestrous period varying from 55 days to 291 days, more in winter months than in summer months, in both the groups. Annual pattern of plasma progesterone showed regular cyclicity during the detected oestrous cycles with peak level from day 10 onwards depending upon the length of the cycle. Low level of nutrition in cows of group II led to long anoestrous periods which were also, as a result of winter season an another triggering factor.

7. Influence of pre, peri and post partum conditions on serum calcium, phosphorus and Magnesium in Buffaloes

S.A. QUAYAM, S.R. PATTABIRAMAN, J. G. DEVANATHAN

Madras Veterinary College—Madras 7.

Variation in the concentration of serum calcium inorganic phosphorus and magnesium during pre, peri and post-partum Murrah buffaloes was studied and compared. Serum calcium concentration was observed to be more in primiparous than in pluriparous buffaloes during pre partum period. During peri-partum both calcium and phosphorus showed sharp decline and then increased gradually during post partum period. On the contrary the magnesium concen-

tration increased during peri partum and then declined by 60 days post partum.

Calcium phosphorus ratio of 2.3:1 in primiparous buffaloes at 60 days pre-partum increased to 3.25:1 at the time of parturition and continued to be maintained till 60 days post partum. In pluriparous buffaloes the calcium phosphorus ratio was 2.15:1 at the time parturition. Subsequently the ratio became narrow upto 45 days and then became wider by 60 days post partum.

8. "Studies on the influence of mineral biochemical and haematological levels at 5 day peripartum on the occurrence of postpartum oestrus in buffaloes"

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To find out the relationship between the mineral, biochemical and haematological values at 5 days peripartum to the occurrence of postpartum oestrus, mean values of the minerals (calcium, phosphorus and magnesium), biochemical (Serum total proteins and blood glucose) and haematological (Hb, PCV MC MC) constituents at 5 days peripartum of those pluriparous buffaloes that showed oestrus at 60, 90, 120, 150 and 180 days were compared with the values of corresponding anoestrus groups were observed except that inorganic phosphorus level in 60 days oestrus group was significantly

more than in anoestrus group. Blood glucose concentration was observed to be significantly higher at 60 and 90 days in oestrus than anoestrus groups. Serum total protein concentration also did not differ significantly between oestrus and anoestrus groups. Haematological values at 5 days peripartum did not show significant variation among oestrus and anoestrus groups at different post-partum periods. In primiparous buffaloes minerals, biochemical and haematological values in the oestrus groups were not significantly different with those of anoestrus groups.

9. Certain Haematological variations in pre, peri and post partum conditions in buffaloes

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Blood samples from 35 pluriparous and 19 primiparous Murrah buffaloes were subjected to assess Hb, P.C.V. and MCHC values from 60 days pre partum through peripartum and upto 60 days post partum at fortnightly intervals. In primiparous buffaloes haemoglobin and PCV levels were strikingly more

than that of pluriparous animals. At the time of parturition the values declined sharply and then during post partum period they tended to remain almost at same level. Haemoglobin and packed cell volume in primiparous buffaloes were almost similar to the value observed in pluriparous animals.

10. Studies on serum progesterone levels in relation to occurrence of uterine torsion in Buffaloes

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Serum samples from 32 buffaloes suffering from uterine torsion approximately at the completion of gestation period were analysed for progesterone concentrations by radioimmunoassay. Fifteen buffaloes had progesterone concentrations more than 1.00 $\mu\text{g/ml}$ ($1.65 \pm 0.13 \text{ ng/ml}$; 1.10 to 2.80 ng/ml) while the serum progesterone concentrations in rest 17 buffaloes were less than 1.00 $\mu\text{g/ml}$ ($599 \pm 59 \text{ pg/ml}$; 200 to 900 pg/ml). The occurrences of uterine torsion were associated with labours and/or abdominal pains around the expected time of

parturition on completion of gestation period. High progesterone concentrations at labour in torsion cases in almost half of the buffaloes might suggest that these labours were premature resulting from impaired hormonal milieu. Disturbances in onset of labour owing to hormonal imbalance as evidenced by high progesterone levels might as well contribute in the causation of uterine torsion.

Cases of uterine torsion having low progesterone values have also been discussed.

11. Characters of semen and lactic acid profile at different hours of preservation in Jamnapari and Barbari Bucks

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The study was conducted on a total of 68 semen samples collected twice weekly from 5 Jamnapari and 3 Barbari bucks of approximately same age and kept under similar managerial regime. Semen samples were assessed immediately after collection for various seminal characters such as volume, initial motility, live sperm percentage and sperm concentration. To determine the lactic acid content of the neat semen, one ml of breedwise pooled semen was taken and deprotenized immediately. Breed differ-

ences for various seminal characters were studied. Significant effect of breed ($P < 0.01$) was noted on motility percentage and sperm concentration in the neat semen. In diluted semen the effect of breed was also found to be significant ($P < 0.01$) on motility percentage and percentage of live spermatozoa at 0, 48, 72 and 96 hours of preservation. Lactic acid content had negative significant correlation with percentage of motile and live spermatozoa in both the breeds.

12. Profile of sex steroids and thyroid hormones in relation to development and reproductive performance of male crossbreds

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Forty Seven crossbred calves and bulls were used. Of these 22 animals were classified into three age groups and the rest of the breeding bulls were grouped under three categories as good, medium and poor depending on their semen quality, serum samples from these animals were analysed for testosterone, oestradiol-17B. Progesterone thyroxine and triiodothyronine by Radioimmunoassay. The testosterone level in growing animals increased from 0.2 ± 0.04 ng/ml at 1-3 months of age to 6.11 ± 1.17 ng/ml at 1.3 years of age. The oestrogen and progesterone also recorded a little increase with age. Bulls donating good

quality semen (motility more than 75%) had lower concentration of testosterone (9.67 ± 2.42 ng/ml) than the bulls donating poor quality of semen (motility less than 30%). The oestradiol-17 B concentration was also higher in bulls donating poor quality of semen than those donating good quality semen. The thyroxine was quite high (84.00 ± 18.14 ng/ml) in the bulls donating poor semen, whereas, it was comparatively low (67.37 ± 7.39 ng/ml) in the bulls donating good quality of semen. The results showed that very high levels of peripheral hormones particularly testosterone has a deleterious effect on seminal quality.

13. Transaminases in cattle & Buffalo bull semen Vis-A-Vis fertility and seminal characteristics during moderate and colder seasons

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Semen from eight (4 cattle and 4 buffalo) bulls was collected and analyzed for physical characteristics and the transaminase activity. The mean values of glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) activity in the whole semen and the seminal plasma were 1566.6 ± 63.43 and 367.6 ± 25.07 , and 806.8 ± 30.63 and 121.2 ± 10.85 SF units, respectively for cattle and 1359.4 ± 47.96 and 278.6 ± 78.78 , and 634.5 ± 28.28 and 94.2 ± 11.01 for buffalo. In cattle the mean semen volume was 5.7 ml (range 0.3-10.0), mass activity 3.5 (0-5 scale), and sperm concentration

1402.1 millions per ml (range 170-2880); whereas in the buffalo it was 3.3 ml (range 0.5-7.0), 2.8 (0-5 scale) and 1028.5 millions per ml (range 210-1850) respectively. Cattle semen had significantly ($P < 0.01$) higher mass motility, sperm concentration and transaminase activity as compared to buffalo. The semen quality, in general, tended to be better during moderate ($15-24^{\circ}\text{C}$) season compared to the colder one ($9-14^{\circ}\text{C}$). The enzyme activity was positively correlated with mass motility, sperm concentration and fertility.

14. Comparison of physiobiochemical semen characteristics in normal and problem bulls

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A study on "Andrological Investigation of nine cross-bred bulls" was undertaken during a period of one year covering cold (Nov-Feb.), hot (Mar-June) and wet (July-Oct) seasons. This included evaluation of seminal characters, assay of seminal biochemical constituents and estimation of haematological parameters. Out of nine bulls studied two bulls (22.22%) were problem bulls. On detailed clinical andrological examination, seminal, biochemical and histopathological studies, these two problem bulls were diagnosed to have testicular hypoplasia.

The clinical findings in seven normal and two problem bulls for comparison were: scrotal circumference 34.93 ± 0.72 vs 30.75 ± 0.51 cm, testicular volume 1037.59 ± 47.65 vs 837.50 ± 23.72 ml, R-test 1164.81 ± 100.81 vs 425.00 ± 17.68 and sperm survivability in cervical mucous 1.96 ± 0.20 vs 0.84 ± 0.21 respectively. Further in normal and problem bulls:

- (a) The seminal characters were: sperm motility 3.68 ± 0.08 vs 1.43 ± 0.13 , sperm concentration/ml

1121.77 ± 36.58 vs $653.75 \pm 90.48 \times 10^6$, abnormal sperm per cent 5.76 ± 0.37 vs 38.73 ± 0.37 .

- (b) The biochemical constituents of semen were: initial fructose 540.59 ± 19.38 vs 393.18 ± 95.58 mg %, citric acid 386.73 ± 76.83 vs 352.86 ± 29.84 mg %, total sialic acid 53.30 ± 1.31 vs 39.95 ± 7.44 mg %, total protein 10.29 ± 0.13 vs 7.68 ± 0.35 g % and inorganic phosphorus 14.59 ± 0.45 vs 14.14 ± 3.27 mg %.
- (c) The results of mineral assay in semen samples through EDAX (dry wt %) were: Ca: P ratio 0.397 vs 0.822, Na: K ratio 0.627 vs 0.345, sulphur 29.727 vs 15.509, chloride 22.240 vs 42.104, copper 7.821 vs 4.380 and zinc 3.394 vs 1.567 respectively.

Problem bull Rosy 8-14 had low germinal cell resistance hypoplasia and subsequent testicular degeneration and the bull Deolo having bilateral total testicular hypoplasia as confirmed on histopathological examination.

15. Prepubertal spermatogenic pattern and sperm defects

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The primary sperm defects have been attributed due to disturbances of spermatogenesis. Abnormal spermatogenic pattern in hamster during prepubertal life was examined to investigate the origin and nature of some defects of sperm structure in the cauda epididymis.

The morphological features of spermatozoa recovered from the hamster epididymis have been examined. Data are presented on the frequencies of several different morphological classes of sperm

defects during prepubertal period. The findings show the existence of a strikingly high incidence of abnormal forms in animals examined 5 to 6 weeks after birth and there is clear evidence of a decrease in the frequency of these forms in the succeeding week of life. The more striking malformations were observed in the structure of the head and middle-piece.

The morphological features of the sperm defects shall be demonstrated.

16. Studies on Certain Biochemical Parameters of Semen of Jersey F₁ and F₂ Crosses

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In order to fix a definite percentage of blood, the criss-crossing have to be adopted. This could be done by keeping F₁ and F₂ Crosses. Scanty reports are available for semen quality of F₂ Crosses. It was with this view that the present investigation was carried out.

Nine adult comprised of experimental animals, three each of Jersey, F₁ (Jersey × Sahiwal) and F₂ (Holstein Friesian × Jersey × Sahiwal) Crosses. These animals were kept under uniform husbandry

condition of feeding and management. The ejaculates were taken during rainy season. Each animal was allowed to donate semen once weekly for six consecutive weeks. The neat semen was evaluated initially by noting the physical characteristics.

The biochemical constituents such as total protein, fructose, lactic acid, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), inorganic phosphorus, acid soluble phosphorus, acid

phosphatase, sodium and potassium were determined in the fresh semen.

The biochemical constituents like total protein and potassium did not show any significant difference in different groups of animals. Fructose showed significantly higher values in Jersey than F_2 Crosses, showing that utilizable substrate for the live spermatozoa was available in sufficient quality in Jersey as compared to F_2 Crosses. Lactic acid production was more in F_1 as compared to F_2 . Inorganic phosphorus was more in F_1 as compared to

other bulls. It was correlated significantly with acid soluble phosphorus and sodium in F_2 Crosses. It was further correlated with acid soluble phosphorus in F_2 Crosses only, clearly indicating that the phagocytic activity in semen of F_2 Crosses did not involve high energy phosphate. Lactic acid was significantly positively correlated with sodium in F_2 Crosses indicating that low level of lactic acid in F_2 Crosses as compared with others, was depressed by the sodium.

17. Testosterone profile of poor semen quality bulls.

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and

R.J. Dash, Department of Endocrinology, P.G.I. Chandigarh

Testosterone levels were estimated by following RIA technique from normal and poor semen quality bulls of various genetic bases. The screening and classification of the bulls was done on the basis of physical parameters of semen quality. It was observed that testosterone concentration in bulls having 50% or less progressive motility and more than 20% dead

spermatozoa, was less than 2.0 n mol/l. No significant difference was noticed in testosterone levels within bulls of normal and poor semen quality groups. However there was significant difference within individuals of same genetic group with respect to its level. A positive correlation was established between testosterone levels and semen quality.

18. Effect of cyproterone acetate on rat epididymal enzymes

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In a prelude to use cyproterone acetate as castration agent among scrub breeding bulls, a study was conducted on 25 male Albino rats. Each rat except 5 controls was administered 50 mg/kg. B.W/IM of cyproterone acetate daily until 3 Wks, 6 Wks, 9 Wks and 12 Wks, respectively, from 30 days of its life. Some of the important enzymes like lactic dehydrogenase, succinic dehydrogenase, alkaline phosphatase, Acid phosphatase and Esterase were estimated from epididymal

fluid of sacrificed animals. The results indicated an overall decline in the concentration of all the enzymes from the C.A. treated group as compared to control animals irrespective of the period of administration. However, the decrease was more pronounced in young rats than in the matured ones and C.A. became less effective as the age advanced. It was, therefore, concluded that C.A. if given at an early stage of a male may hamper the reproductive processes.

19. Studies on certain enzymes in the epididymis of local goats.

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Enzymes of various kinds abound in the epididymal plasma. In the present study the fluid from DE (Ductuli efferentis), Caput, Corpus and Cauda was studied from 18 mature local bucks. The mean values for GOT, GPT, acid and alkaline phosphatases were observed to be 104.10 ± 3.00 , 3.58 ± 1.17 , $151.33 \pm$

5.92 and 121.66 ± 10.13 in DE, 131.66 ± 5.83 , 6.66 ± 0.41 , 226.66 ± 3.33 and 171.33 ± 11.33 in Caput, 109.16 ± 1.16 , 7.91 ± 0.41 , 140.00 ± 11.45 and 156.66 ± 10.47 in Corpus and 211.66 ± 3.67 , 10.41 ± 1.10 , 287.66 ± 12.66 and 313.00 ± 14.43 in Cauda, respectively.

20. Cholestrol and 17-Ketosteroids levels in blood serum and testes of cyproterone acetate treated rats.

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Livestock Research Station, Gujarat Agricultural University,
Campus Junagadh (Gujarat)

Cholestrol and 17-ketosteroids were estimated from serum and testicular fluids of one month old male Albino rats when administered cyproterone acetate at a rate of 50 mg/kg BW/I.M daily to 6 wks. The mean values for cholestrol and 17-Ketosteroids in testis and serum of treated and control animals

were 0.877 ± 0.05 , 0.730 ± 0.064 , 57.16 ± 1.17 , 60.28 ± 3.37 mg/100 ml and 28.79 ± 0.60 , 17.96 ± 0.66 , 13.36 ± 0.64 and 8.21 ± 0.64 and 8.21 ± 0.31 mg/100 gm, respectively. The data indicated enhanced levels of Cholesterol and 17-ketosteroids in these tissues on cyproterone acetate administration except serum Cholesterol.

21. Total lipid & cholesterol content of cervicovaginal mucus of Jersey cows during estrous cycle and early pregnancy

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The total lipid and cholesterol contents of cervicovaginal mucus of Jersey cows showed cyclic variation during different stages of estrous cycle. 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 estrous phase and from 66.7 to 74.7 with a mean of 71.6 ± 4.9 during non-estrous 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 cholesterol content (mg/100 g, wet

wt, mean in parenthesis) ranged from 0.108 to 0.120 (0.113 ± 0.001) during estrous and 4.849 to 4.865 (4.857 ± 0.002) during non estrous 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. Significant differences were recorded during non-estrous phase of pregnant and non-pregnant groups and it was concluded that these parameters can form a basis of diagnosing early pregnancy in cattle.

SESSION: VII

Efficacy of drugs on induction of Oestrus

1. Comparative efficacy of oestrus inducing drugs in dairy animals under field conditions.

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1712 non cyclic breedable dairy population constitute the basis of study. The pupulation was spread over eight treatments namely, ovarian massage, lugols iodine paint on cervix, capsules (Herbogyne), cocu tablets with lugols paint, enucleation of CL, mineral mixture with lugols iodine, vitamin A and D3 and mineral mixture. The response to different treatment was measured as the effect on oestrus exhibition and conception. It was evident that the enucleation of corpus luteum was found to be highly effective in restoration of oestrus (79%) in village animals followed by vitamin A and D3 (62%), Capsules (52%), lugols iodine paint on cervix (51%), mineral mixture and lugols iodine (47%), cocu tablets and lugols paint (46%), mineral mixture (44%) and ovarian massage

(40%). However, a divergent trend was observed in conception. The higher conception was attributed to the treatment of mineral mixture with lugols iodine paint (70%) followed by capsules (60%) cocu tablets with lugols paint (57%), lugols paint on cervix (56%), vitamin A and D3 and ovarian massage (54%), enucleation of corpus luteum (33%) and mineral mixture (27%). The study suggested that a combination of mineral mixture and lugols paint on cervix for a period of three weeks is more effective, in conceiving anestrus dairy animals under field conditions. This was an exploratory study on a small population of dairy animals under field condition. Further, trials in different regions, breeds, blood traits and species are suggested.

2. Induction of Estrus in Cows using Prostaglandin $F_{2\alpha}$ via intra-vulvosubmucous route.

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Ten randomly selected cyclic cows (group I) were given 5 mg Prostaglandin $F_{2\alpha}$ (PG) via intravulvosubmucous

route between 8-12 days of their estrous cycle. Another three cows with persistant corpus luteum (group II) were also

administered PG via the same route. All the cows in group I returned to estrus within 144 ± 30 hrs (Mean \pm SE) of PG administration. Seven of the ten cows (70%) conceived to first insemination. The remaining three cows were diagnosed as suffering from endometritis and were treated accordingly. Two cows conceived to the subsequent insemination.

Three cows of the group II took invariably longer time (168 ± 12 hrs; mean \pm SE) to return to estrus. None of the cows in this group conceived to first insemination. However, only one cow conceived to second insemination. It is suggested that intravulvosubmucosal injection of PG is a reliable and economic way of inducing estrus in cattle.

3. Ovarian response to Prostaglandin and three continuous administration of Gondotropins in cows.

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Synchronization of oestrus was done with $\text{PGF}_{2\alpha}$ (Estrumate-Miles Lab. U.S.A.—2 ml. containing 500 mcg-cloprostenol) i.m. in 6 cross-bred normal lactating cows. 100% of cows came in heat on 3rd day. Ovulation occurred in 100% cows treated with LH (Chorulon-Intercare, India-10 ml. containing 3000 i.u.) i.m. whereas only 33.33% cows ovulated with only $\text{PGF}_{2\alpha}$ treatment.

Experiment on Superovulation was done on 3 crossbred cows which were

treated with $\text{PGF}_{2\alpha}$ i.m. (500 mcg-cloprostenol) followed by Gonadotropin i.m. on 13th, 14th and 15th day (Folligon-Inter Care, India-3000 i.u.). The dose of $\text{PGF}_{2\alpha}$ was repeated on 15th day to induce ovulations. On an average 15 well developed large size (>2 cm) follicles were observed in each animal on 17th day but there were no ovulations. Administration of LH (Chorulon-Inter Care, India, 3000 i.u.) i.m. on 18th day helped only 24.4% follicles to ovulate.

4. Amphoteric effects of Bromocriptin plasma FSH Concentrations in the Anoestrous and oestrous ewe.

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Administration of bromocriptine (B), a dopaminergic receptor agonist stimulated the FSH release in the anoestrous ewe, dissociated the synchronous surges of FSH and LH, and caused an early FSH surge in the oestradiol-treated anoestrous ewe. B (given at 2 h after the onset of oestrus, AOE) inhibited the second FSH surge in 3 of 4 ewes. B (given at 0, 12, 24 and 36 h after cloprostenol, C, injection on Day 10 of the oestrous cycle) inhibited the pro-oestrous FSH release

in 3 of 5 ewes and significantly blocked the FSH surge. B did not affect the time of ovulation and conception in ewes given B at 0 or 12 h after C, or at 2 or 18 h AOE. Delayed ovulation occurred when B was given at 36 h or at 0, 12, 24 and 36 h after C.

The present results suggest the amphoteric effects of B depending upon the physiological status of the reproductive phases.

5. Effect of "bovisynchron" therapy on hormonal levels in cross breeds of red-dane SRB and Jersey exotic inheritance

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"Bovisynchron" product of VEB--Jena Pharma Serum work, Bernburg, GDR, was tried on five cross bred heifers, which attained the puberty age (average 600 days) and weighed (average 170 kg), but did not express any estrus for studying the hormonal levels of FSH, LH and Prolactin and for synchronisation of estrus in these animals.

Gynaeco-clinical examination was done and blood-serum samples were collected, prior to during and after treatment, and

on synchronised estrus states, for estimation of FSH, LH and Prolactin levels by RIA technic.

FSH, LH and Prolactin levels varied highly significantly, during and following treatment and as well as on synchronised estrus.

The highly significant rise in FSH, LH and Prolactin might have brought the desired effect of inducing and synchronisations of estrus in 80 per cent cases.

6. Synchronization of oestrus and fertility with Prostaglandin F₂ alpha in Cross Bred Cattle.

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The study on synchronization of oestrus and fertility with prostaglandin F₂ alpha i.e. Iliren (Hoechst) revealed that 26.66, 53.33 and 20 percent oestruses were synchronized within 48 hours, 48 to 96 hours and more than 96 hours with single injection regime while with double

injection regime 75 and 25 percent oestruses were synchronized within 48 hours and 48 to 96 hours after the last injection. The fertility at first synchronized oestrus was 38.46, 62.5 and 46.66 percent in group A, B and control respectively.

7. Clinical and Hormonal studies on Synchronization of estrous in goats with chronogest intravaginal sponges.

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Fifteen, beetal goats, aged between 3 to 4 years and 45 and 60 days post kidding were implanted with Chronogest (Chronolone-40 mg) intravaginal sponges for synchronization of estrus. The sponges were left in situ for 14 days. Each animal received 400 I.U. of Folligon (PMSG) at the time of Sponge removal intramuscular. The animals were handmated at 48 and 60 hours after removal of the spiral. All the animals exhibited excellent signs of estrus 48 hours post sponge removal. The animals were examined for preg-

nancy and followed till kidding. Blood samples were collected by Jugular venipuncture at day 0 (pre spiral implantation) and on days 1, 3, 5, 7, 9, 11, 13 and 14, and 48 and 72 hours post spiral removal. The plasma progesterone concentrations were studied by Radio-immunoassay. The paper discusses the fertility, fecundity and prolificacy, as well as the plasma progesterone concentrations in the synchronized animals.

8. Effect of low dose of $\text{PGF}_{2\alpha}$ on luteolysis in cattle

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Holstein cows during mid-luteal phase were injected with 125 μg Cloprostenol ($\text{PGF}_{2\alpha}$ analogue) through intramuscular route into gluteus muscle ipsilateral (Gr. I, $n=26$) or contralateral (Gr. II, $n=16$) to the ovary having corpus luteum or at the neck (Gr. III, $n=7$). Twenty three (88.5%), 10 (62.5%) and none (0%) of the treated cows in Gr I, II and III, respectively came in oestrus and ovulated. Oestrus symptoms were generally pronounced in animals responding to the treat-

ment. Effectiveness of the dose when injected into the gluteus muscle and its failure on injection being made at the neck region indicate the transport of Cloprostenol from the site of injection to ovaries through countercurrent transfer from veins to arteries in and around pelvic areas. The luteolytic dose of Cloprostenol in cows can, thus, be reduced from 500 μg recommended dose to 125 μg if injected into the gluteus muscle.

SESSION: VIII

Super ovulation and Cytogenetics

1. "Studies on superovulation and morphological evaluation of Embryos in Goats"

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Following determination of the ovulation rate of adult Black Bengal goats at spontaneous oestrus (2.8 ± 0.2), superovulation was attempted in 12 goats with 1000 I.U. PMSG administered on day 10-12 of the oestrous cycle, followed 48 h later with 100 μ g of a PGF₂ α analogue. Six of these animals additionally received 1000 I.U. HCG i.v. at oestrus. The animals were hand mated at 12 h intervals during standing oestrus. Ovarian response was determined by directly visualizing the ovaries at laparotomy.

An average of 12.0 ± 2.49 ovulations were recorded in animals treated with PMSG & PGF₂ α . Additional HCG neither improved the superovulatory response (10.5 ± 3.19) nor reduced the individual variability. Its beneficial use in increasing the proportion of mature follicles ovulat-

ing was also not evident.

Embryo collection was attempted on day 3, 4 or 5 in superovulated goats. The mean recovery rates for days 3 and 4 were quite high (75 to 80 per cent), but declined drastically (<20 per cent) when attempted on day 5.

76.25 per cent of the recovered ova were classified as fertilized, developing to approximately 4, 6 and 12 cell stages on days 3, 4 and 5 respectively. A high proportion (75.4 per cent of the fertilized ova exhibited normal cleavage and morphology.

Early returns to oestrus were noticed in some superovulated goats, however, this phenomenon was less pronounced in animals receiving HCG. Post-operative fertility was within the normal range.

2. Effect of induced single or multiple ovulations on biochemical and enzyme constituents of oestrous mucus in cattle.

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Twenty-two dairy cows and heifers maintained under uniform conditions of feeding and management were divided into two treatment groups. Animals in first group (Single Ovulation SO group) were treated with 500 μ g cloprostenol

as intramuscular injection on 9th day of a normal oestrous cycle. Animals in second group (Multiple Ovulation-MO group) were given 1500 I.U. serum gonadotrophin on 9th day of a normal oestrous cycle and 48 hr later treated

again with 500 µg cloprostenol. Each experimental animal was examined during pre-treatment, induced and cyclic oestrous periods and oestral cervical mucus for biochemical analysis was collected from all the animals during the three oestrus periods and were analysed for acid and alkaline phosphatase activity, protein, total reducible sugars, sodium and potassium.

In the SO group, the induced oestrous mucus did not show significant variation in the levels of phosphatases, protein total reducible sugars, sodium and

potassium from that of pre-treatment and cyclic oestrous mucus. In MO group, serum gonadotrophin-cloprostenol treatment resulted in significant increase in alkaline phosphatase activity and increased phosphatase activity, total reducible sugars and potassium levels remained almost the same as in pre-treatment and cyclic oestrous mucus. In induced oestrus of MO group the alkaline phosphatase activity, protein and sodium levels were significantly higher than that of SO group.

3. Effect of repeated superovulation on ovarian response and non surgical embryo collection from cows.

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Ranchi Veterinary College, Ranchi

Superovulation was attempted to study the rate of response following repeated intramuscular administration of 1500 I.U. of PMSG (Antex, Leo) and 25 mg of PGF₂ alpha (Lutalyse, Up John) in six crossbred (Friesian X Local) dairy cows. The animals were inseminated twice after superovulation at 12 hours interval with chilled semen from bulls of documented fertility 48 hours after second dose of PGF₂ alpha. Repeated superovulation was performed at every 8-10 weeks using the same treatment regimen. The experiment was conducted over a year such that all the cows were superovulated for six times at regular intervals. Ovarian monitoring, on the day of embryo collection (D 8-12) revealed good (>10 CL), Moderate (6-10 CL)

and poor (<6 CL) response in three, one and two animals respectively. Embryo collection was performed with the help of Foley's catheter and Phosphate Buffer Saline was used as flushing medium. No Corpus Luteum could be palpated in the poorly responding animals at first attempt, however, a good number of anovulatory follicles were palpated. Subsequent treatments revealed presence of a few corpora lutea in them. 54.1 percent of the ovulated ova in 34 attempts of superovulation could be recovered. The rate of ovarian response decreased significantly ($P < 0.01$) at the subsequent treatments. The recovery rate of fertilized ova was observed to be about 47 percent.

4. A modified method for assessment of embryos.

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Sixteen 2-4 cell embryos collected from the oviduct of superovulated does. These embryos were kept in basal salt solution with antibiotic in sterile condition at 0-4°C for 24 hours. The embryos were exposed to UV for 1 hour to be certain of their death. Twelve 2-4 cell embryos were collected freshly. Both the two types of embryos were kept in 20 ml of Culture fluid mixed with 0.2 ml of 2% Trypan blue for 30 minutes.

Trypan blue entered into all the sixteen embryos of the 1st group and

became blue in colour, whereas none of the freshly collected embryos of IIInd group took any dye.

On culture the group II embryos were found to be growing whereas embryos of group I did not grow as they were dead.

It may be inferred that trypan blue may be used to assess the survival or dead embryos for transfer, to curtail down the *in vitro* culture, prior to transfer for the assessment of live or dead embryos.

5. Nuclear anomalies of erythrocytes & leucocytes among the bulls with poor libido & poor semen quality.

V. SHANKER, S. BHATIA

Scientist S-2, N.D.R.I., Karnal

Thirty seven bulls belonging to Sahiwal (5), Karan Swiss (19) and Karan Fries (13) breeds and exhibiting poor libido and poor semen quality were screened for sex chromatin and nuclear anomalies of erythrocytes and leucocytes. Among these animals nine failed to donate semen due to absence of libido, 23 showed poor libido and poor semen quality and 5 exhibited inconsistent semen quality. Examination of the blood smears of these animals revealed singularly or in combination several kinds of nuclear anomalies of leucocytes and erythrocytes. Five animals were found to show drum-stick positive neutrophils in their blood

smears. Various anomalies of polymorphonuclear leucocytes included abnormal nuclear lobulation, presence of unsegmented nuclei and leucocyte nuclei with well delineated chromatin free light areas. Fall in number of blood polymorphonuclear leucocytes was also observed in several animals. The erythrocytic anomalies observed among these animals were cytoplasmic projections and or central stroma. However, ten bulls with normal libido and normal semen quality showed absence of the abnormalities of leucocytes and erythrocytes in their blood smears.

6. Anatomical and cytogenetical studies on a XX/XY chimeric goat

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Chimerism is the admixture of the haemopoietic cells originating from different zygotes. This is also an identification tool for freemartinism in bovines which are habitually uniparous. However, it is rarely observed in multiparous animals such as the goat. Authors found a XX/XY chimeric goat with a typical reproductive organs.

The Beetal horned goat with typical male appearance and male bleat had a very big bean shaped clitoris (1.4 cms) with cork shaped protuberance of (0.6 cm). Sex chromosomes were studied in 83 metaphase plates by lymphocyte culture method. It had 77.11% (64) XX and 22.89% (19) XY cells and thus was chimeric.

The goat was a member of quadruplet set of two males and two females. Two of its co-twins (a male and a female) were not chimeric while the fourth co-twin (male) was not available for study.

Autopsy was performed after slaughtering the goat. Udder tissue was found

to be poorly developed. The testicles were found not at the inguinal region but at the usual site of ovaries. There were no ovaries. The blood supply was also typical of the testes. There was a commissure between the two presumed testes which were later confirmed by histological examination. Fallopian tube like structures were descending from testes and were followed by "horns". The "horns" descended parallel and did not join to make body of the "uterus". However, these got fused in a very short "uterus". A small cervix was present. There were seminal vesicles around the junction of two "horns" (uterus and cervix). The vagina was of usual size. There was a female type external genital opening with a big clitoris. There are a few published reports on chimeric goat freemartins but the present case has a very unusual bisexual reproductive anatomy. The presumptive etiology will be discussed.

7. A quantitative study of germ cells in prenatal buffalo ovary

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Quantitative study of germinal cell population was undertaken in the ovary of Buffalo foetus. The highest number of germ cells were found at day 121-150 (7020.28 per 0.0176 sq. mm), although the weight of ovary increased at 6 month, where decrease in weight and surface area of ovary was associated with reduction in cell population. The volume of germ cells was found maximum (473468.6043) in near-term ovary with corresponding reduction in the cell

count and it was attributed to the formation of primordial and growing follicles. The percentage of normal degenerating germinal cells was highest (1016.40%) during last stage of gestation. During this study three waves of germ cells degeneration was recorded at 3, 4 and 6 months of gestation. The ovarian germ cell activity revealed active oogonia at early, while abundant active oocyte/primordial follicle at late stages of gestation.

8. Sex chromalin studies in cattle: Constancy of drumstick absence among reproductively inefficient animals from birth to maturity

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In a routine screening of 90 young female calves (Karan Swiss and Karan Fries crossbred strains) for sex chromatin anomalies, ten animals were observed to show absence of drumsticks in the polymorphonuclear leucocytes of their blood smears. These animals also exhibited several specific nuclear anomalies of polymorphonuclear leucocytes and erythrocytes otherwise lacking in normal animals. On tracing these animals till their maturity they showed stunted growth (5), late maturity (3) and repeat

breeding (2). The examination of their blood smears at the time of culling revealed once again absence of the drumstick appendages and also the presence of various anomalies of leucocytes and erythrocytes. These observations indicated that the animals detected to show anomalous sex chromatin patterns and also other morphological abnormalities of their blood cells early in their life continued to possess these anomalies till their maturity and exhibited disturbed fertility in their later life.

SESSION: IX

Physio Chemical Properties and Deepfreezing of Semen

1. Seminal characteristics and deep-freezing of cross-bred bull semen using tris-yolk glycerol extender.

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Volume, mass activity and sperm concentration were studied in seminal ejaculates of Holstein Friesian \times Haryana (HF \times H), Brown Swiss \times Haryana (BS \times H) and Jersey \times Haryana (J \times H) Bull of approximately same age and maintained under standard uniform feeding and housing management. These semen samples (12 from J \times H, 7 from HF \times H from BS \times H) were also deep-frozen in mini straws (0.25 ml capacity) using tris yolk glycerol (TY-G) extender with 4 hours equilibration time. Sperm motility was recorded after extension, equilibration time and deep-freezing. There was non-significant differences in volume and mass activity of semen among different crosses while sperm concentration differed significantly ($P < 0.05$), between J \times H and HF \times H bulls and J \times H and BS \times H bulls. However, sperm concentration between HF \times H and BS \times H bulls, did not differ significantly. Sperm motility was higher in case of HF \times H bulls

(67.86 ± 1.86 per cent), followed by J \times H (64.17 ± 1.03 per cent) and BS \times H (59.29 ± 1.70 per cent), differences being significant ($P < 0.05$) among different crosses. Equilibration did not alter the motility pattern of spermatozoa from pre-equilibration state. The post-freezing (Post thaw) motility of spermatozoa was significantly ($P < 0.05$) higher in HF \times H bulls (52.14 ± 2.40 per cent) than in J \times H (43.33 ± 1.86 per cent) and in BS \times H (42.14 ± 3.24 per cent). However, the differences in post-freezing motility between J \times H and BS \times H bulls was not significant. Positive but non-significant correlations between various physical characteristics (volume, mass activity and sperm concentration) and post-freezing motility was also observed. The loss in motility between initial extension and deep-freezing of semen was 15.72, 17.15 and 20.84 per cent, respectively in HF \times H, BS \times H and J \times H bulls spermatozoa.

2. Seminal characters, Freezability & Fertility in Mehsani and Murrah buffaloe bulls

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Eighty four semen collections from seven healthy Mehsani and ninety six semen collections from eight Murrah buffalo bulls were studied for a period of one year. The buffalo bulls were on 2-3 ejaculates a week collection schedule and were under uniform conditions of management & nutrition. Tris-egg yolk-fructose-glycerol was used with single step glycerolization & equilibration time of 4-5 hours at 5°C.

Mean ejaculate volume (ml.), initial motility (%), sperm concentration (millions)/ml. sperm concentration (millions)/ejaculate, pH, 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 & 8.33 ± 0.25 respectively. These values for Murrah buffalo bulls were: 3.84 ± 0.17 , 77.23 ± 0.24 , 1341.12 ± 19.88 , 5312.18 ± 154.4 , 6.83 ± 0.02 , 9.58 ± 0.16 and 7.55 ± 0.17 respectively. Semen quality was poor during hot months of summer & improved as rainy season advanced with a fall in ambient temperatures.

Mean post-thaw motility (%), losses during freezing (%) and—conception rate (%) for Mehsani buffalo semen were: 56.26 ± 5.45 , 21.0 ± 1.47 & $44.82 \pm$

0.38, respectively. These values for Murrah buffalo semen were: 53.97 ± 1.08 , 23.26 ± 1.13 & 44.69 ± 0.77 percents respectively.

10.57% of 473 semen ejaculates & 11.03% of 435 semen—ejaculates from Mehsani & Murrah buffalo bulls were found to be either flat or of poor freezability.

Freezability and conception rates were observed to be superior during the cooler months of September to February in both the breeds.

The number of artificial inseminations performed and the conception rates obtained with liquid buffalo semen ranged from—16268 to 23174 and 29.18 to 31.80% during the years 1975-81, which increased to 36102 to 69328 inseminations and 37.50 to 44.91% conception rates during 1981-85 when the frozen semen was used for insemination.

The liquid semen doses produced per buffalo bull per year varied between 3151.54 to 3801.62 whereas 5111.42 to 6881.12—frozen semen doses per buffalo bull per year could be produced—indicating better utilization of the buffalo bulls with frozen semen technique.

3. Studies on the freezability and revivability of the cross bred bull semen in three different extenders

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Seventy two ejaculates were collected from 4 Holstein-Friesian \times Kankrej cross bred bulls and sixty six ejaculates were collected from 4 Jersey \times Kankrej cross bred bulls. Ejaculates were divided into 3 equal parts and extended in Egg Yolk Citrate (EYC), Egg Yolk Lactose (EYL) and Tris Fuctose Yolk Glycerol (TFYG) and frozen on liquid nitrogen vapour in straws. Thawing of frozen semen in EYL consistently gave a better recovery rate of spermatozoa than EYC and TFGY in both the cross-bred bulls. Percentage

of post thawing motility of spermatozoa in EYC, EYL and TFGY were 34.63 ± 1.23 ($32.03 \pm 0.03\%$), 41.30 ± 1.10 ($43.6 \pm 0.04\%$) and 34.99 ± 1.33 ($32.9 \pm 0.05\%$), in HF \times K cross-bred bulls and 31.93 ± 1.25 ($28.0 \pm 0.05\%$), 40.32 ± 1.27 ($41.9 \pm 0.05\%$) and 34.61 ± 1.33 ($32.3 \pm 0.05\%$) in J \times K cross-bred bulls, respectively.)

The results indicated the better efficiency of EYL for freezing of bovine spermatozoa.

4. Post thaw motility and longevity of buffaloe spermatozoa in different extenders

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Twenty ejaculates, collected from five Nili-Ravi buffalo bulls, were split into three parts and diluted in different extenders, i.e., Lactose, Lactose-fructose and Tris-tes-fructose. The straw method of deep freezing was used. After thawing, survival rate was recorded until the death of all spermatozoa. The initial post-thaw motility and survival of spermatozoa were 32.75% (25-47.5%), 5 hrs; 20% (2.5-37.5%), 4 hrs; 14.75% (zero-32.5%), 3 hrs in Tris-tes-fructose, Lactose-fructose and Lactose extenders respectively. Statistical analysis revealed a highly

significant difference between diluters regarding post-thaw motility of spermatozoa ($P < 0.01$) and Tris-tes-fructose was significantly better than other two extenders ($P < 0.01$). However, the difference between bulls and bull \times extender interaction were found to be insignificant. Present data suggests that Tris-tes-fructose may be a suitable diluent for preservation of buffalo semen. Moreover, studies on comparative evaluation of other extenders for freezing of buffalo semen need to be conducted.

5. Freezability and post thaw semen characteristics of Bucks of different breeds

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Eleven bucks representing three breeds, Jamunapari (4), Barberi (4) and Black Bengal (3) were included in this study. Semen was collected by means of artificial vagina twice a week. Volume, colour, mass activity and percentage of live sperm were recorded before freezing. Semen samples were then prediluted in Tris extender with 10% volume of egg yolk. The samples were centrifuged at 3000 rpm for 15 minutes. The supernatant was discarded and the centrifugate was rediluted with Tris extender containing

20% egg yolk. After packaging in 0.3 ml Landshut straws the samples were allowed different hours of equilibration, viz., 2h, 4h, 6h, 20h at 4-6°C. Samples were frozen in Liquid Nitrogen vapour. Difference with regard to freezability was observed between breeds, bucks and ejaculates. Effect of hours of equilibration was evident in post thaw characteristics of semen which included motility, percentage of live sperm, percentage of abnormal sperm and percentage of damaged acrosomes.

6. Efficacy of tris-citric acid and sodium citrate buffers against cold shock to buffalo spermatozoa

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Sperm motility, live and abnormal spermatozoal and GOT enzyme were recorded before and after cold shock in buffalo semen, extended in tris—Citric acid and Sodium citrate buffers. The difference in percent motile spermatozoa before cold shock was not significant but after cold shock the per cent motile spermatozoa were significantly ($P < 0.01$) higher in tris—citric acid (28.00 ± 4.33) than in Sodium citrate buffer (18.00 ± 6.74). The per cent live spermatozoa before cold shock were more (65.40 ± 3.05) in tris-citric acid buffer and less in sodium citrate (56.80 ± 3.20), the difference being significant ($P < 0.01$). However, after cold shock, these differences became non-significant. The extent of post shock sperm abnormalities too

did not differ significantly either due to buffers or due to cold shock. The release of GOT before cold shock was significantly ($P < 0.01$) less in tris-citric acid (74.50 ± 4.25 units/ml) than in Sodium citrate buffer (110.50 ± 10.05 units/ml). Although, the total release of GOT after cold shock to semen extended in tris-citric acid buffer was comparatively less (111.50 ± 14.07 units/ml), as compared to sodium citrate buffer (130.00 ± 7.15 units/ml), but the difference was not significant. Based on the above results it may be considered that for deep-freezing of buffalo semen, tris-citric acid buffer with optimum level of egg yolk, cryoprotective agents and antibiotics can be used successfully.

7. Effect of thawing time on semen frozen in french straws

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In the practice of artificial Insemination the information as to how long the freeze thawed semen could be used for breeding of animals is important. Studies were undertaken to evaluate the influence of field handling procedures on the survival rate and fertilizing capacity of the spermatozoa. Semen samples which were frozen and stored for long time were taken at random, out of the Liquid Nitrogen container. They were kept at (a) body temperature (38°C) (b) room temperature (25—30°C) and (c) in Ice water (5°C) up to a period of 75 minutes. The motility, live percentage, and acro-

somal integrity were estimated at periodic intervals. It was observed that reasonable motility was maintained up to 45 minutes when the straw were kept at a constant temperature of 38°C followed by keeping in Ice water. The longevity of spermatozoa was found to be considerably low when kept at room temperature. Two hundred and nineteen animals were inseminated after 45 minutes of thawing of which 39 cows repeated within a period of 60 days of insemination. This indicates that thawing period up to 45 minutes do not effect the fertilizing capacity of the spermatozoa.

8. Effect of post thawing preservation on motility and fertility of bull semen

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One hundred and twenty eight ejaculates obtained from three Jersey and two Jersey cross-bred bulls were used to study the effect of post thawing preservation on motility and fertility of bull semen. Semen were extended in skim milk (SM) and egg yolk citrate (EYC) extenders and after five hours of equilibration period it was frozen in 0.5 ml French straws and stored in liquid nitrogen. The straws, after thawing at 37°C for 10 seconds were kept in a test tube, wrapped with non-absorbant cotton inside a wide mouth thermos flask containing ice cubes. The motility of spermatozoa at 0, 1-2, 2-3 and 3-4 hours of preservation

was respectively 64.08, 63.56, 63.10 and 62.74 percent for SM extended semen and 68.09, 67.41, 67.09 and 66.72 percent for EYC extended semen. There was significant difference ($p < 0.01$) in mean motility of spermatozoa between SM and EYC extenders in all the periods of preservation. The motility did not differ significantly between preservation periods in both the extenders.

The fertility rate did not drop significantly upto 1-2 hours of post thawing preservation. But fertility rates obtained at 2-3 hours and 3-4 hours of post thawing preservation were significantly lower than that obtained at 0 hour.

9. Effect of storage at -196°C on quality of goat semen frozen with and without seminal plasma in tris based extender

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Ten ejaculates, two from each of 5 local bucks, were frozen in 0.5 ml French straws with and without seminal plasma in Tris egg yolk citric acid fructose glycerol extender by rapid horizontal vapour freezing technique. The percentages of progressively motile and live sperm and damaged acrosomes in frozen semen were determined at '0' day (14 hours), after 3, 6, 9 and 12 months of storage in liquid nitrogen (-196°C). The mean percentages of progressively motile and live sperm did not vary significantly between storage periods, between treatments (With and without seminal

plasma) and due to storage period \times treatment interaction. The percentage of damaged acrosomes in semen frozen with and without seminal plasma was 11.20 ± 1.55 and 14.80 ± 1.21 at '0' day and 16.30 ± 1.48 and 19.30 ± 1.23 after 12 months of storage. The percentage of damaged acrosomes differed significantly ($P < 0.01$) between storage periods, between treatments (with and without seminal plasma) but did not differ significantly due to storage period \times treatment interaction. The increase in percentage of damaged acrosomes was significant only after 6 months of storage.

10. Effect of diluters on motility and fertility of frozen goat semen

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Twenty four ejaculates, 12 from each of two Beetal bucks were used to study the relative efficacy of egg yolk citrate fructose glycerol (EYCFG) and Tris egg yolk citric acid fructose glycerol (TEYCAFG) diluters for freezing of buck semen. The ejaculates were diluted at the rate of 1:10 with the two diluters by split sample technique. The diluted semen was cooled from 37°C to 5°C in 1.5 hours. After 4 hours of equilibration at 5°C , the semen was vapour frozen in 0.5 ml. French straws. Thawing of semen was done in water at 37°C for 10 seconds. The percentage of progressively motile spermatozoa (PPM) in EYCFG and TEYCAFG diluters was 75.20 ± 1.10 and 78.00 ± 0.65

respectively at prefreezing, and 61.10 ± 1.10 and 64.90 ± 1.05 respectively at post freezing. The PPM decreased significantly ($P < 0.01$) due to freezing in both the diluters. The PPM did not differ significantly between diluters at pre freezing while it was significantly higher ($P < 0.05$) in TEYCAFG diluter at post freezing.

Of 40 and 52 does inseminated with semen frozen in EYCFG and TEYCAFG diluters, 38 and 52 does respectively were followed up for 60-90 days non-return. The non-return rate for the two diluters was 78.95 and 88.46 per cent respectively, the difference being non significant.

11. Keeping quality of frozen thawed chilled Buffalo semen

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Deep Freezing of buffalo semen was done in Tris-Egg yolk dilutor with 6% Glycerol. The semen was packed in French mini (0.25 ml) and French medium (0.50 ml) containing 40 million spermatozoa. The Frozen semen thawed at two temperatures Viz. +5 °C. for 2 minutes and +37°C. for 30 seconds was preserved as chilled semen at +5°C. and the revival of spermatozoa in respect of progressive motile percentage was studied at 0 hr., 4 hr. & 8 hr. of preservation.

The overall progressive motile spermatozoa percentage observed in French mini straw ($38.78 \pm 3.06\%$) was signi-

ficantly superior to French medium straw ($35.46 \pm 3.14\%$) in chilled semen. The revival rate of spermatozoa at thawing temperature of +37°C. ($43.77 \pm 1.15\%$) was significantly higher than that of +5°C. ($30.47 \pm 1.15\%$). The mean progressive motile percentage of freeze-thawed-chilled semen differed significantly between 0 hr. & 4 hr.; 4 hr. & 8 hr. and 0 hr. & 8 hr. The study indicates that freezethawed (+37°C.)-chilled semen can very well be used for insemination upto a period of 8 hrs. of storage at +5°C. in the field where it is not possible to maintain the straws in LN2 containers.

12. Deep freezing of buffalo semen in different packing straws, freezing rates, and thawing temperatures

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The revival rate of Frozen buffalo spermatozoa in Tris Egg Yolk dilutor with 6% Glycerol was studied in different packing straws, Viz. French mini (0.25 ml.), French medium (0.50 ml.) and German Land Shut (0.25 ml.) containing 40 millions spermatozoa; freezing rates, Viz. Conventional (4 cms above LN2 level) and Rapid (2 cms above LN2 level) and thawing temperatures Viz. +37° for 30 seconds & +55°C. for 12 seconds. The revival rate in respect of progressive motile % observed by Rapid ($53.45 \pm 1.22\%$) freezing was significantly superior to that of conventional ($48.44 \pm 0.91\%$) freezing method. The revival rate of spermatozoa was

significantly higher at thawing temperature at +55°C. ($52.57 \pm 1.49\%$) than that of +37° ($49.32 \pm 1.24\%$). The revival rate of spermatozoa in French mini ($52.57 \pm 1.85\%$) and German Land Shut ($52.15 \pm 1.94\%$) was found significantly superior to French medium straw ($48.55 \pm 1.43\%$).

The decrease in SDH activity was also less in freeze thawed semen in French mini and German Land Shut straw as compared to French medium straw. Thawing at +55°C. for 12 seconds indicates lesser damage to spermatozoa as evinced by decrease in percentage of SDH activity.

13. Studies on influence of thawing temperature and medium on Post thaw Physiological changes of buffalo semen

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In the present investigations an attempt has been made to study the effect of different thawing media—Atmospheric air at 18°C for 6 minutes and in water at 20°C for 90 seconds and at 40°C for 30 seconds on post thaw physiological changes of buffalo semen frozen in two diluents; Tris and Egg-yolk citrate. Certain physical parameters like motility, non-eusinoiphilic count and morphological abnormalities and biochemical parameters like Hyaluronidase, GOT and GPT enzymes were studied. Post

thaw incubation studies were also conducted on the above mentioned Physiological parameters to know how long the thawed semen could be used with useful motility.

The results have indicated the buffalo semen extended in Tris diluent and thawed at 40°C for 30 seconds in water would be more suitable than the other media and temperatures studied. Post thaw incubation of buffalo semen at 37°C would facilitate the use of buffalo semen for longer periods (3 hours) after thawing for AI in buffaloes.

14. Effect of thaw rates on motility, survival and acrosomal integrity of buffalo spermatozoa frozen in medium french straws

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The objective was to determine the effect of different thaw rates on motility, survival and acrosomal integrity of buffalo spermatozoa frozen in medium french straws. Sixteen ejaculates from four mature buffalo bulls of Murrah breed were tested in a 4×4×4 factorial combination. Semen was extended in Tris egg yolk glycerol extender, frozen in 0.5 ml polybinyll chloride straws in liquid nitrogen vapour and stored in

liquid nitrogen for 24 h. Straws were thawed at water bath temperatures of 30°, 37° or 75°C for 30s, 15 or 30s, and 9s respectively. Semen was incubated at 37°C for 6 h and evaluated at hourly intervals for percentage of motile spermatozoa (% MOT), percentage of total spermatozoa with intact acrosomes (PIA) and percentage of spermatozoa with intact, healthy acrosomes (PIHA) after 0 and 3 h of incubation. The initial post-

thaw motility (Oh) average 66.9, 66.6, 72.1 and 64.6% for the four thaw rates respectively. Differences were significant between thaw rates for % MOT at 0 h ($P<0.05$) and 1 h ($P<0.01$) evaluation, post-thaw sperm survival at 37°C and absolute index of sperm survival. Bulls also differed ($P<0.01$) for % MOT at 1, 2, 3 and 4 h evaluation, post-thaw sperm

survival at 37°C and absolute index of sperm survival. Significant ($P<0.01$) interaction of thaw rate \times bull for % MOT at 1 h evaluation was observed. Either treatments or bulls had any significant effect on PIA and PIHA after 0 and 3 h incubation. Thaw rate of 37°C for 30 s was comparatively superior to other rates studied.

15. Fertility of chilled and deep frozen buffalo bull spermatozoa under field conditions

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In order to evaluate the fertility of Buffalo spermatozoa deep frozen in medium french straws, data on 3009 Buffalo—Cows in 16 field A.I. units which were verified for pregnancy following first A.I. with semen from 4 Murrah Bulls has been obtained and analysed. Semen was extended in Tris egg yolk glycerol extender to contain 30 million spermatozoa per straw equilibrated for 6 hours, frozen in 0.5 ml polyvinyl chloride straws in liquid nitrogen vapour for 8 minutes by rapid horizontal vapour freezing technique and stored in liquid nitrogen for varying periods. Inseminations were done by experienced technicians during later half of the heat period by using A.I. gun and later verified for pregnancy by rectal palpation 60—90 days post-insemination.

The overall first conception rate was 50.9%. Between Bull and between season variation in conception rate was significant. ($P<0.05$) During summer

season, the conception rate was significantly less (47.5%) when compared to winter and rainy seasons.

Data were also obtained on first service conception rate of 1645 buffalo-cows inseminated with chilled semen from the same four bulls, extended in Egg Yolk citrate dilutor, preserved at 4 degree centigrade and supplied to these A.I. Centres prior to introduction of frozen semen. The overall first service conception rate obtained with chilled semen was 47.1%. This was significantly ($P<0.05$) less than that obtained with frozen semen. Bulls varied in conception rate while seasonal fertility differences were non significant. Long distance transport of chilled semen of Buffalo bulls was found to have adverse effect on fertility.

It is concluded that the use of frozen semen improved first service conception rates of buffaloes as compared to chilled semen in field conditions.

16. Deep freezing of bull semen in soluble gelatin containers

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A novel method of packing and storage of semen was standardised. 0.5 ml. of diluted bull semen (Diluted in egg Yolk tris glycerol dilutor) was filled in shellac coated gelatin containers (Size "0"). 3 Pure bred Jersey and 3 Holstein-Friesian bulls, and 3 each of Jersey \times Gir crosses (50:50) were included for the trial.

This innovative mode of packaging and storage of semen was compared with

the existing straw method. Parameters like Progressive Motile Percentage, Vital count, Abnormal percentage and Acrosomal Intactness were studied comparatively before and after freezing for both the methods.

Sixty ejaculates were processed and the results were found to be fairly good for storage of semen in gelatin containers under deep freezing conditions.

17. Freezability of bovine spermatozoa in laiciphos-478 employing "Tupol" technique

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Several extenders have been tried on ultra-low temperature preservation of bovine spermatozoa viz. egg yolk citrate, egg yolk lactose, tris, skim milk etc. with success. A variable degree of success have been reported for the preservation of buffalo spermatozoa at ultra-low temperatures in skim milk, egg yolk citrate, citric acid whey and tris.

The Laiciphos-478 (France) has been used at this Institute for the preservation of cattle and buffalo spermatozoa at ultra-low temperature and its efficiency has been compared with egg yolk citrate. A total of 40 ejaculates from four bulls (two Holstein Friesian and two buffalo) were taken for this experiment. Consequently ten ejaculates from each bull were fractionated into the two aliquotes after the assessment of its quality diluted in

EYC and Laiciphos-478. The level of glycerol at 7% and equilibration time of 4-6 hrs were maintained for both the extenders.

The average post-thawing percentages of Holstein-Friesian bulls semen (No. HF 166 and HF178) were 45.0 ± 2.24 and $49.0 \pm 2.3\%$ in Laiciphos-478, whereas the corresponding values were 31.0 ± 1.80 and 33.0 ± 1.86 in egg yolk citrate.

The average recovery rate of buffalo semen (buffalo No. 849 & 8) were 35.0 ± 2.89 and 36.0 ± 2.77 in Laiciphos-478 whereas the values were 25.0 ± 2.24 and 24.0 ± 1.64 in egg yolk citrate. Highly significant differences were recorded in pre-freezing and post-freezing percentages. A significant difference was recorded in the recovery rate of the two extenders.

18. Migration rate of unfrozen and frozen semen of holstein friesian bulls in the oestral mucus of cows

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Samples of oestral mucus were collected from 67 non descript cows during the follicular phase of the oestrus cycle. Uterine and Ovarian changes were monitored by rectal palpation and accordingly the cows were grouped as having 1st, 2nd and 3rd degree of uterine tone. The migration rate of sperms in the mucus was recorded by Sprojte syringe. Levels of sodium and potassium were determined by Systronic Flame Photometer. The migration rate of sperm in the oestral mucus depended on the

tonicity of the uterus and level of electrolytes in the mucus. The migration rate of unfrozen sperms was recorded to be 50.6 ± 1.1 mm/20 min. and of frozen sperms 57.3 ± 1.2 mm/20 min. Sodium level was found to be 294.6 ± 5.9 mg/100 ml and that of potassium was 62.7 ± 2.2 mg/100 ml. Levels of sodium and potassium and migration rate of unfrozen and frozen sperms of Holstein Friesian bulls in oestral mucus collected at different degrees of uterine tone were found to be significant ($P < 0.01$).

SESSION: X

Anoestrous and Miscellaneous

1. Reproductive performance of unproductive dairy animals following induction of lactation

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Estradiol-17 β and Progesterone dissolved in ethanol were given subcutaneously twice daily at 12 hrs interval for seven consecutive days to initiate lactation and changes in reproductive performance in non-lactating and non-pregnant dairy animals (9 cows and 9 heifers). The daily dose of estradiol and progesterone was 0.1 mg and 0.25 mg/kg body weight respectively.

First estrus behaviour was noted on

3.85 ± 0.59 and 3.55 ± 0.41 days in heifers and cows respectively after the 1st injection. Regular estrus appeared on 74.4 ± 3.76 and 87.6 ± 2.87 day in 5 heifers and 5 cows (55.55%) and 4 heifers (44.44%) and 2 cows (22.22%) conceived and calved normally. Statistically a difference ($P < 0.05$) was observed on days required for appearance of regular estrus between two groups of animals.

2. Effect of feed additives on milk production and silent heat problems in dairy cows

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Directorate of Veterinary Services, West Bengal

A product containing certain amino acids, vitamins and minerals have been tried on Dairy cows having problems of low milk yield in a group and silent heat in another. It has been found that use

of the product could positively augment milk production and induce heat, resulting in pregnancy in the former and latter groups respectively.

3. Effect of laparoscopy on reproductive performance in sheep

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Central Institute for Research on Goats, Mathura

Effect of laparoscopy on fertility was studied in ewes. The ewes were laporoscoped for various diagnostic purposes during oestrous cycle. Laparoscopies were conducted once, 2-3 times, 4-5 times or more than 6 times in a group of 37 ewes.

Another sub. group not laparoscoped at all served a control. Laparoscopy upto 5 times per oestrous cycle did not effect fertility. The body weights of pregnant ewes during gestation did not vary between groups.

4. Induction of lactation in infertile cattle histological and ultrastructural studies:

M.A. NOWSHAHRI and S.N. MAURYA

Department of Gynaecology and Obstetrics
College of Veterinary Science
G.B. Pant University of Agriculture & Technology
Pantnagar (India)

The histological and ultrastructural changes were investigated in mammary parenchyma of infertile cattle artificially induced to lactation. The lactation was induced by administration of diethylstilboestrol dipropionate @ 7 mg/100 kg body wt and progesterone @ 20 mg/100 kg body wt for 12 days, prednisolone @ 20 mg/animal/day on day 14,15 and 16th and reserpine on day 17 to day 19. Tissue Biopsies were obtained from 10 animals once in an animal on day 0,16 and 35th of the therapy. Milking was commenced on day 20 of the treatment period.

The highly dense or loose areolar connective tissue prevalent on day 0 was replaced by alveolar tissue on day 16

and further increase was noticed on day 35 in cattle successfully induced to lactation while as involuntary changes were observed in non-successfully induced cattle on day 35. Electron microscopy revealed well developed organelle and secretory activity in successful cattle on day 35 but in unsuccessful cattle convoluted nuclei surrounded by a thin band of cytoplasm were seen. The mammary development was not equivalent to that of a normal post parturient cattle even in successful cattle on day 35. The results suggest that more emphasis should be directed towards achieving the cell proliferation and differentiation for the development of mammary gland equivalent to that of a normal post parturient cattle.

5. Some observations on female and male genital systems of montijac deer (*Muntiacus muntijac*)

K.K. BONIA and H.K. GOGOI

Department of Animal Production & Management,
College of Agriculture, Assam Agril. Univ.,
Jorhat—785013 (Assam)

By the department of Forest, Diphu, Government of Assam, a female and two male Montijac deer—popularly known “Barking deer” (*Muntiacus muntijac*) were found as unclaimed carcasses and were handed over for a report. With due

permission of the authority concerned, the genital system of these animals were subsected for a gross anatomical and biometrical observations followed by recording photographically, numerically and discussed accordingly.

6. Angioarchitecture of uterus and histology of uterine artery in different reproductive conditions in buffaloes

S. KUNCHITHAPATHAM, and S.R. PATTABIRAMAN,

Madras Veterinary College, Vepery—Madras 600 007

Radiographic technique with 20 per cent lead oxide solution as radio opaque material was employed to study angioarchitecture of normal, pathological, anestrus and gravid uterus in buffaloes.

The middle uterine arteries on either side of the uterus were inter connected with one to three prominent anastomising arches at the level of the body of the uterus. The uterine branch of the vaginal artery joined the middle uterine artery and also formed distinct anastomising arch at the level of the cervix. From these arches fine arterial net work ramify in the midline along the intercornual ligament. The arcuate arteries were thin and less coiled in anestrus condition, much

reduced in length and indistinct in perimetritis and straight and thicker in gravid uterus. The body of the gravid uterus was distinctly free of arcuate arteries. One or two uterine branches of ovarian artery were observed to anastomose with the middle uterine artery.

In gravid uterus the uterine artery showed either subendothelial thickening, splitting and fragmentation of the lamina elastica interna or proliferation of endothelial cells. In uterus with perimetritis the uterine artery showed subendothelial thickening of tunica intima and splitting of lamina elastic interna resembling arteriosclerosis.

7. The ischiorectal fossa approach in a cow for the surgical treatment of an abscess in the uterine wall

D. JOHN and J. RAJASEKARAN

Madras Veterinary College

A successful surgical treatment of an abscess in the uterine wall is described. The uterus is exteriorised through the ischio rectal fossa. This has given better visualisation, all possible exploration and treatment without complication of peritonitis etc. The bleeding is negligible. It is quite safe. Animal had an uneventful recovery and became pregnant in the same horn. The other possible uses of this approach are also discussed in the report.

Abscess in the uterine wall in cows has been reported by Roberts (1982) and this has to be differentiated from a tumour, cyst or hematoma. He has also

reported one case caused by improper use of an artificial insemination pipette and several cases aborting at the fourth month of pregnancies on successive gestations and sold as sterile animals. According to him treatment is usually impossible without the danger of causing severe perimetritis or peritonitis. If the abscess is small the animal may recover but in many cases the animal becomes sterile.

A successful surgical treatment with the uterus approached through the ischio rectal fossa followed by pregnancy is reported.

8. Experimental aflatoxicosis and testicular histology

B. NEDUNCHERALATHAN, R. SRINIVASAN, D. KATHIRESAN and S.R. PATTABIRAMAN

Madras Veterinary College Madras-7

Since there exists contradicting reports on the effect of aflatoxicosis of testicular histology, some favouring testicular degeneration due the affection and some others indicating no ill effect controlled experiments were conducted to assess the influence of the aflatoxicosis on testicular histology. Guinea pigs and rabbits were the subjects of the study. Litter mates were divided into control

group and the experimental group. Experimental group guinea pigs were fed with 0.5 ppm of AFB₁ and the experimental rabbits were fed with 0.3 ppm of AFB₁ for 18 weeks and 16 weeks respectively. Histopathological studies of the testis of the animals sacrificed at 4, 9 and at the end of 16 or 18 weeks revealed that the chronic aflatoxicosis has no ill effects on testicular histology.

9, Estradiol-17 β and Progesterone dependent changes in the serotonin level of Pineal of ovariectomized hens

S. KAJAL and R.P. MOUDGAL

Department of Animal Production Physiology,
Haryana Agricultural University,
Hisar—125 004, India

Serotonin (5-HT) in Pineal was determined after an hour of last injection (I/M) administered in two combinations of 17 β —estradiol and Progesterone (10+50 μ g and 50+500 μ g/kg. body weight) for three consecutive days in the ovariectomized hens. Estradiol-17 β together with Progesterone (10+50 or 50+500 μ g/kg

body weight) caused significant drop in 5-HT content of Pineal of ovariectomized hens (0.167 ± 0.032 or 0.256 ± 0.025 μ g/mg) in comparison to that in control group (0.408 ± 0.42 μ g/mg). This observations suggested that gonadal hormones are capable of modifying the levels of 5-HT of Pineal gland.

10. 'Serotonin in Pineal of chicken in relation to follicular status'

S. KAJAL and R.P. MOUDGAL

Department of Animal Production Physiology,
Haryana Agricultural University,
Hisar—125 004, India

Serotonin (5-HT) in Pineal was estimated at the age of 24 weeks in three groups of hens categorized based upon the individual ovarian follicular status (white embeded follicles but no yellow follicle, yellow follicles but no empty follicles and yellow follicles with empty follicles). A decreasing trend in the concentration of 5-HT in Pineal gland was observed in the hens approaching earlier sexual maturity in comparison to late maturing hens of same age group. The hens with yellow and empty follicles were found to have significantly lower concentration of 5-HT (0.142 ± 0.017 μ g/mg) in the Pineal

than that of hens having only White embeded follicles (0.297 ± 0.009 μ g/mg). The values in the hens having yellow follicles (rapid phase of development) but no empty follicles (0.197 ± 0.022 μ g/mg) fell in between the values of other two groups, without having any significant difference with either of the groups. This observation suggests that either the lower levels of 5-HT in Pineal might have some useful role to play in triggering the gonadal maturity or the higher concentration could be playing an inhibitory role in the development of ovary.

11. 'Serotonin in Pineal of ovariectomized and sham-operated hens around sexual maturity'

S. KAJAL and R.P. MOUDGAL

Department of Animal Production Physiology,
Haryana Agricultural University,
Hisar—125 004, India

Serotonin (5-HT) in Pineal gland was quantified, between 09.30 to 10.30 hours, at the age of 14, 18, 20, 22 and 26 weeks in sham-operated and ovariectomized hens. It was found significantly more in ovariectomized hens than in sham-operated ones at all the age groups. In both ovariectomized and sham-operated groups, decreasing trend in 5-HT concentration with the advancement of age around the sexual maturity was noticed. A

significant difference was noted between 20(0.402 ± 0.031 $\mu\text{g}/\text{mg}$) and 22 weeks (0.223 ± 0.027 $\mu\text{g}/\text{mg}$), and 22 and 26 weeks (0.137 ± 0.023 $\mu\text{g}/\text{mg}$) of age in sham-operated. The values of 5-HT also varied significantly at 20 (0.672 ± 0.030 $\mu\text{g}/\text{mg}$), 22 (0.580 ± 0.087 $\mu\text{g}/\text{mg}$) and 26 weeks (0.372 ± 0.054 $\mu\text{g}/\text{mg}$) of age in ovariectomized hens. These observations indicated inverse relationship between 5-HT and gonadal development.

12. Relationship between different stages of Milk Production in Bhadawari Buffaloes

B.N. VERMA, H.B. DWIVEDI, R.P. TIWARI, M.P. KATIYAR, C. SINGH

C.S.A. University of Agriculture and Technology, Kanpur 208002

The mean variability and relationship of various production traits related to different seasons were recorded and found that the shishir calving was best for high yield of colostrum and first fifteen days milk yield and Basant calver for high peak yield and total lactation milk yield. In Greesham season calvers, first colostrum yield as well as peak yield was lowest. This variation is due to the season of calving. The days required to reach peak yield and the persistancy of peak yield, the Hemant calvers reached peak yield earliest while Basant calvers latest. Sharad calvers maintained the persistancy of peak yield longest and the

Basant calvers shortest. The relationship between first colostrum yield and first 15 days milk yield were found to be highly significantly correlated for the calvers of sharad, Hemant and Varsha and significantly correlated for shishir calvers. Similarly the colostrum yield was highly significantly correlated with peak yield for Varsha and Sharad seasons and with total lactation yield only for Varsha season calvers and there was no significant or negative significant correlation for calvers of other seasons. First fifteen days milk yield and peak yield and total lactation yield were highly significantly correlated for Varsha and

Sharad calvers while no significant correlation was found for calvers of other seasons. The peak yield and total lactation milk yield were found to be highly significantly correlated for Varsha and Sharad calvers, significantly correlated for shishir and Basant calvers and non significantly correlated for the calvers of

Greesham and Hemant. It was concluded from the study that total lactation yield can be estimated in Bhadawari buffaloes to a certain extent by knowing colostrum yield for calvers of Varsha, first 15 days milk and peak yield for calvers of Varsha and Sharad seasons.

13. Annular Dissection for reduction of rectum and cervicovaginal prolapse: A case report

M.J. DESHMUKH

District Deputy Director of Animal Husbandry, Jalna

The buffalo of seven years was brought to the Veterinary Polyclinic with a prolapsed mass of rectum and cervicovaginal prolapse. The buffalo had calved about three weeks before. All efforts were tried to reduce the prolapse by routine method and by applying roap truss. It was found of no use, as such the surgical intervention by annular dissection was considered and found successful.

As the case was brought late, the spasm of sphincter may interfere with the blood supply to the prolapsed tissue and become oedematous and cyanotic. Due to excessive straining and increased intra-abdominal pressure vaginal prolapse also occurs along with rectum. The prolapse of rectum was about six inch of the rectal wall which was found completely necrosed.

Performed operation under epidural anaesthesia. The area was prepared in usual manner, cotton is packed in rectum to prevent the passage of faeces. Reten-

tion sutures were given to the prolapsed portion of rectum. The longitudinal incision was made through the m.m. from ano-rectal line to the apex of prolapse. Then circular incision was made through m.m. at each end of longitudinal incision. By blunt dissection the m.m. separated from healthy underlying tissue. Controlled by blunt dissection the m.m. separated from healthy underlying tissue. Controlled the apparent haemorrhages. The procedure was continued until the m.m. was removed.

The continuous sutures with catgut were taken in close contact the circular line and rectal wall. Removed the retention sutures. Automatically the rectal prolapsed portion was set in, and the cervicovaginal prolapse was reduced manually and very conveniently.

In this way the case was observed for five days and treated symptomatically. The case was completely cured without any pursestring sutures.

14. Functional Anoestrus therapy in pubertal Crossbred heifers with progesterone primed Oestrogen

UMA SHANKER, S.K. AGARWAL and L.P. NAUTIAL

Livestock Production Research (Cattle)
Indian Veterinary Research Institute,
Izatnagar, U.P.

Forty functional anoestrus crossbred heifers having above 225 kg body weight and 15 months and above age were assigned to four treatments: Untreated control; 200 µg oestradiol Benzoate intramuscularly; 0.5 mg MGA per head daily orally for 10 days; M.G.A. 0.5 mg for 10 days followed by Oestradiol benzoate 200 µg injected 48 hr. after the last day of MGA feeding. In respond, in control group 20% heifers exhibited natural ovulatory oestrus and also conceived simultaneously. In the treated group 30, 50 and 80% heifers ovulated at the induced heat with OB, Melengestrol acetate (M.G.A) and M G A + OB treatment. The conception rate in

induced oestrus was 10, 20 and 50% in O B, M G A and M G A + OB treatment groups. The subsequent cycle length after induced oestrus was 16.0 (50%), 17.0 (33.3%) and 18.0 ± 0.3 (100%) days in O.B., M.G.A. and M G A + OB treated animals only, 1 (50.0%), 1 (33.3%) OB and M G A treated heifers could not show the subsequent cycle after induced oestrus. In the first natural cycle all the treated heifers were conceived. These results indicate that ovarian cyclicity and ovulatory oestrus can hormonally be induced in functional anoestrus pubertal crossbred heifers for early breeding with normal age and weight.

15. Studies on Biochemical profiles in Anoestrus Buffalo Heifers

R.K. CHANDOLIA, S.K. VERMA, R.C. GUPTA

Dept. of Gynaecology & Obstetrics, H.A.U., HISSAR

The present study was conducted on thirty two anestrus and twenty five cycling buffalo heifers maintained at Progeny Testing Farm, Hissar. Cycling buffalo-heifers were kept as control in the present investigation.

No significant variations were observed in blood glucose and total plasma protein levels in anestrus and cycling heifers during estrus and diestrus phases.

Total plasma vitamin E levels were not significantly different between anestrus and cycling heifers during different phases of estrous cycle. The vitamin A

levels were significantly ($P < 0.01$) higher during diestrus phase than those in anestrus and estrus phase in cycling heifers. However, variations in plasma vitamin A levels were found to be non-significant in anestrus and cycling heifers during estrus phase.

No significant variations occurred in plasma calcium levels in anestrus and cycling heifers. However, plasma inorganic phosphorus levels were significantly ($P < 0.01$) lower in anestrus as compared with estrus and diestrus phases in cycling animals.

16. Studies on certain blood constituents of anoestrous cross bred cattle

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Department of Gynaecology, Orissa Veterinary College
Bhabaneshwar 751003

The levels of certain blood constituents were precisely determined to correlate their deficiencies in anoestrous cross bred cattle.

The serum calcium level in anoestrous heifers and cows were 9.42 ± 0.27 and 10.04 ± 0.27 mg.%, which was not significantly different in comparison to control. A significantly lower level ($P < 0.01$) of inorganic phosphorus was observed in anoestrous heifers (4.84 ± 0.19 mg%) and anoestrous cows (4.87 ± 0.27 mg%) when compared to the cycling heifers and cows (control). But the difference in values of anoestrous heifers and cows was not significant. The serum calcium and

inorganic phosphorus ratio was found to be higher in anoestrous heifers, but in anoestrous cows, the ratio was significantly higher ($P < 0.05$). Serum magnesium levels in anoestrous heifers and cows were 2.34 ± 0.06 and 2.41 ± 0.13 mg%, which did not differ significantly. But significantly lower values ($P < 0.05$) of serum protein were obtained in anoestrous heifers (5.89 ± 0.16 gm.%) and anoestrous cows (5.95 ± 0.17 gm.%). The post treatment values revealed significant difference ($P < 0.01$) in inorganic phosphorus, total protein and calcium—phosphorus ratio in cattle exhibiting oestrous.

17. Hormonal and non Hormonal treatment of summer Anoestrous in Buffaloes

PRANEE PORNRATTANAPITAK, Y.G. DUGWEKAR

Punjab Agricultural University, Ludhiana

Investigations were conducted to find out the efficacy of hormonal (Pregnant Mare Serum Gonadotropin-PMSG) (Folligon or Chronogest) and non-hormonal (Clomiphene Citrate) (Fertivet) drugs on induction of oestrus and ovulation in buffaloes during summer. Twenty six buffaloes were used in the study of which, 21 animals received 50 mg progesterone per day for 7 days, followed by 1000 i.u. PMSG (6 animals) or 2000 i.e. PMSG (5 animals) on day 8th. Ten buffaloes received 600 mg clomiphene citrate on day 8th and 900 mg on day 9th. Remaining 5 animals served as control. All the 11 anoestrous buffaloes treated with PMSG exhibited oestrus

within 7 ± 1.84 days after the treatment and all (100%) ovulated. Amongst the 10 buffaloes treated with clomiphene citrate 7 (70%) exhibited oestrus within 4 ± 1.88 days after treatment and 5 of these (73.43%) ovulated. Blood glucose levels significantly increased after treatment of anoestrous buffaloes with progesterone, PMSG or clomiphene citrate. Serum cholesterol, inorganic phosphorus and calcium did not show any significant variation between anoestrous condition and during the oestrous cycle. It is concluded from these studies that both, PMSG or clomiphene citrate, could be successfully used for the treatment of summer anoestrus in buffaloes.

18. Therapeutic use of tonophosphon vet* (Sodium Salt of 4-Diethylamino-2-Dimethylphenyl Phosphonic Acid) in Anoestrus Buffaloes

SATISH KUMAR

Indian Veterinary Research Institute
Izatnagar—243122

Phosphorus plays an important role in metabolism, influences vital body functions and is utilized in bone formation and synthesis of essential compounds of the tissues like phospholipid and nucleic acid. It stimulates the smooth muscle of uterus and promotes the gonadal and genital activity. Tonophosphon vet 20% solution was used in the treatment of anoestrus buffaloes. The anoestrus buffaloes which did not exhibit any sign of oestrus since last 8 months and had atonic uterus and subactive ovaries were given the treatment. Group I was given 3 injections of Tonophosphon (10 ml intramuscular) on alternate days and were fed 50 gms.

of Minimix (Mineral mixture) for 20 days. Group II received only 50 gms. of Minimix for 20 days. The third group (control) received no treatment. 13 (81.25%) out of 16 buffaloes of group I came in heat within an average 22.30 days (6 to 52 days) from the first day of treatment; out of which 10 conceived. In group II seven (46.66%) out of 15 treated buffaloes showed heat within 28.37 days (9 to 46 days) interval from the first day of treatment; out of which only 4 conceived. In the control group (no treatment) two buffaloes out of 10 buffaloes showed heat within 60 days but none conceived.

19. Efficacy of steroid Hormones in the treatment of Anoestrous crossbred cattle

JASWANT SINGH, B.K. SINGH, BALRAJ SINGH, M.P. SINHA

Ranchi Veterinary College Bihar

A combination of estrogen and progesterone (Secrodyl, Glaxo Allenbury's) was tried to induce estrous in crossbred cows and heifers suffering with true anoestrus condition. Different dose schedule of the drug was tried in different groups of anoestrus cattle. Efficacy of the drug to induce estrous (83.33%) was observed to be the best when it was

administered in the dose of 2 ml (5 mg Megesterol Acetate and 0.03 mg Ethinyl Estradiol/ml) intramuscularly followed by 1 ml consecutively for 3 days. In the groups of animals where the drug was administered at the rate of 1 ml daily for four days and 2 ml daily for three days, the percentage of animals responding were 56.31 and 50.00 respectively.

In control group of animals only 22.22 percent evinced estrous in 36.00 ± 4.00 days, whereas in treatment groups the duration between the day of last injection and onset of estrous varied between 5 to 12 days. Normal deviate test revealed significant difference in occurrence of estrous between the treatment groups. Conception rate in induced estrous was found to be highest (72.22%) in the

group of animals where secrodyl was administered in dose regimen of $2+1+1+1$ ml. The colour, appearance, consistency and fern pattern of cervical mucus did not differ between the treated and spontaneously cycling animals during estrous. However, pH and spinnbarkeit value of cervico-vaginal mucus differed significantly ($P < 0.05$) between induced and spontaneous oestrous.

20. Effect of intra-uterine iodine infusions in anoestrous cows with special reference to serum protein bound iodine levels

H.S. BIRADE, V.L. DEOPURKAR, B.R. DESHPANDE and P.M. PUNTAMBEKAR

Department of Animal Reproduction Bombay Veterinary
College, Parel, Bombay

36 cows having post-partum anoestrous for 90 days or more were selected for this study. Prior to the commencement of treatment, all cows were rectally screened to confirm gonadal inactivity. Cows were grouped into four groups and were subjected to different treatments viz. Lugol's iodine 1%, Pivopol 1% Betadine 1% and 4th group was kept

as control. 20 ml of iodine preparations were given intrauterine at the interval of one week-maximum 3 infusions were carried out.

Protein Bound Iodine levels before and after treatment for consecutive three weeks and one level on 21st days post treatment were estimated.

21. Validity of some Biochemical parameters for the assessment of response to gonadoliberin in anoestrus Buffaloes

R.L. DHOBLE, S.K. GUPTA

I.V.R.I., Izatnagar

The availability of gonadotrophin releasing hormone (GnRH) synthetic analogues-gonadoliberin, has promoted a new era in fertility regulation. The present investigation has evaluated the relation between clinical ovarian response to the administration of gonadoliberin in anoestrus buffaloes and some biochemical parameters in blood indicative of metabolic status.

Thirty four anoestrus buffaloes were treated with GnRH (Buserelin acetate (D-Ser(BU+) 6LHRH (1-9) nonapeptide ethylamide, Hoechst, Frankfurt, Germany) aqueous solution 0.0042 ng/ml, administered as a single intramuscular dose of 5 ml.

The mean interval in days from gonadoliberin treatment to the onset of clinicogynaecological response indicated that 3 animals showing expression of oestrus and or conception responded within 7-10 days while 4 animals took

19-30 days. Gonadoliberin compound appeared effective in buffaloes showing clinico-ovarian response. The ovarian events, however, seem to have been triggered only partially in majority animals with no actual manifestations of oestrus.

The biochemical parameters like calcium, inorganic phosphorus, glucose, haemoglobin and thyroid hormone levels in blood were measured in relation to clinical response to treatment. Haemoglobin, calcium, phosphorus, total proteins, albumin, globulin and thyroid hormone levels did not appear related to clinical response. However, a higher albumin-globulin ratio may be related to a superior response to gonadoliberin treatment in buffaloes. The results indicated a possibility of predicting response to GnRH treatment based on blood glucose level in buffaloes.

22. Studies on efficacy of some medicaments in combating post partum anoestrus condition in Buffalo cows

A.K. BANERJEE, R. ROY CHOUDHARY

Department of Clinics Mohanpur (W.Bengal)

125 high yielding graded Murrah buffalo cows from the city khatahs in Calcutta suffering from prolonged post-partum anoestrus condition due to smooth and inactive ovaries were treated with various medicaments to study their efficacies for oestrus induction and conception by natural service.

Gentle massage of ovaries and horns twice a week for a maximum period of four weeks resulted into induction of heat in 33.33% at an average interval of 9.5 days with an overall conception rate in 66.66% animals.

Gentle massage of ovaries in combination with 5% Lugol's Iodine paint on

external os twice in a week for a maximum period of four weeks induced heat in 46.66% animals at an average interval of 8.0 days with an overall conception 85.71% animals.

Oral administration of 300 mg Fertivet tablet (180 mg cisclomiphene citrate and 120 mg trans clomiphene citrate), a product of M/S. Ar-Ex Laboratories Pvt. Ltd., induced heat in 80.0% at an average interval of 5.0 days with an overall conception in 75.0% animals.

Treatment with Aloes compound, an indigenous product of M/S. Alarsin at the dose rate of 15 tabs. per day per

animal for a maximum period of 10 days induced oestrous in 63.33% buffalo cows of which 57.89% conceived.

Treatment with secrolyl injection (10 mg megasterol acetate and 0.06 mg ethenylestradiol in 2 ml), a product of M/S. Glaxo Laboratories (India) Ltd., for two or three successive days induced heat in 80.0% animals within an average interval of 6.5 days with an overall conception in 33.3% animals.

In the control group observed for six weeks without rendering any treatment, only 15.0% buffalo cows came to heat and all conceived by natural service.

23. Studies on incidence of anoestrus in the Murrah grade Buffalo cows (*Bubalis bubalus*) in the city khatahs of Calcutta (W. Bengal)

R. ROY CHOWDHARY, A.K. BANERJEE

Dept. of Gynaecology and Obstetrics, Mohanpur (W.Bengal)

Of 1890 Murrah grade buffalo cows belonging to city khatahs of Calcutta taken up for this study, 961 (50.84%) suffered from prolonged 'reported anoestrous' condition to the extent of average period of 240 days from the date of last calving. 460 such buffaloes were further studied to find out the various physiological causes of their prolonged anoestrous

condition. The incidences of true anoestrosity (No. G.F. or C.L.), sub-functional ovaries (standing G.F.), Missed oestrus (Functional C.L.), heat during examination (silent ovulation), Pregnancy and Persistent corpus luteum (P.C.L.) were observed respectively in 154 (33.47%), 105 (22.82%), 166 (36.08%), 28 (6.08%), 4 (0.86) and 3 (0.65) buffaloes.

DECLARATION

Statement about ownership and other particulars about THE INDIAN JOURNAL OF ANIMAL REPRODUCTION as required under Rules No 8 of the Registration of News papers (Central) Rules 1956.

FORM NO. IV (Rule No. 8)

- | | |
|--|---|
| 1. Place of Publication | Editorial Office:
Dept. of Gynaecology & Obstetrics
Gujarat Veterinary College, Anand |
| 2. Periodicity of Publication | Bi-annual (JUNE & DECEMBER) |
| 3. Printer's Name
Nationality
Address | Anand Press, Gamdi
Anand-388 001 |
| 4. Publishers Name
Nationality
Address | Dr. D. P. Velhankar
Indian
Bombay Veterinary College, Bombay 400 012 |
| 5. Editor's Name
Nationality
Address | Prof Dr SB Kodagali
Indian
Gujarat Veterinary College, Anand-388 001 |

Names and addresses of individual who own the news-paper and partners, share-holders holding more than 1 per cent of the total capital

Official Organ of
THE INDIAN SOCIETY FOR
THE STUDY OF
ANIMAL REPRODUCTION

Regd. No.: Bom. 253/78

Office:

Dept. of Animal Reproduction
Bombay Veterinary College Parel,
Bombay-400 012

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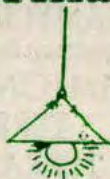
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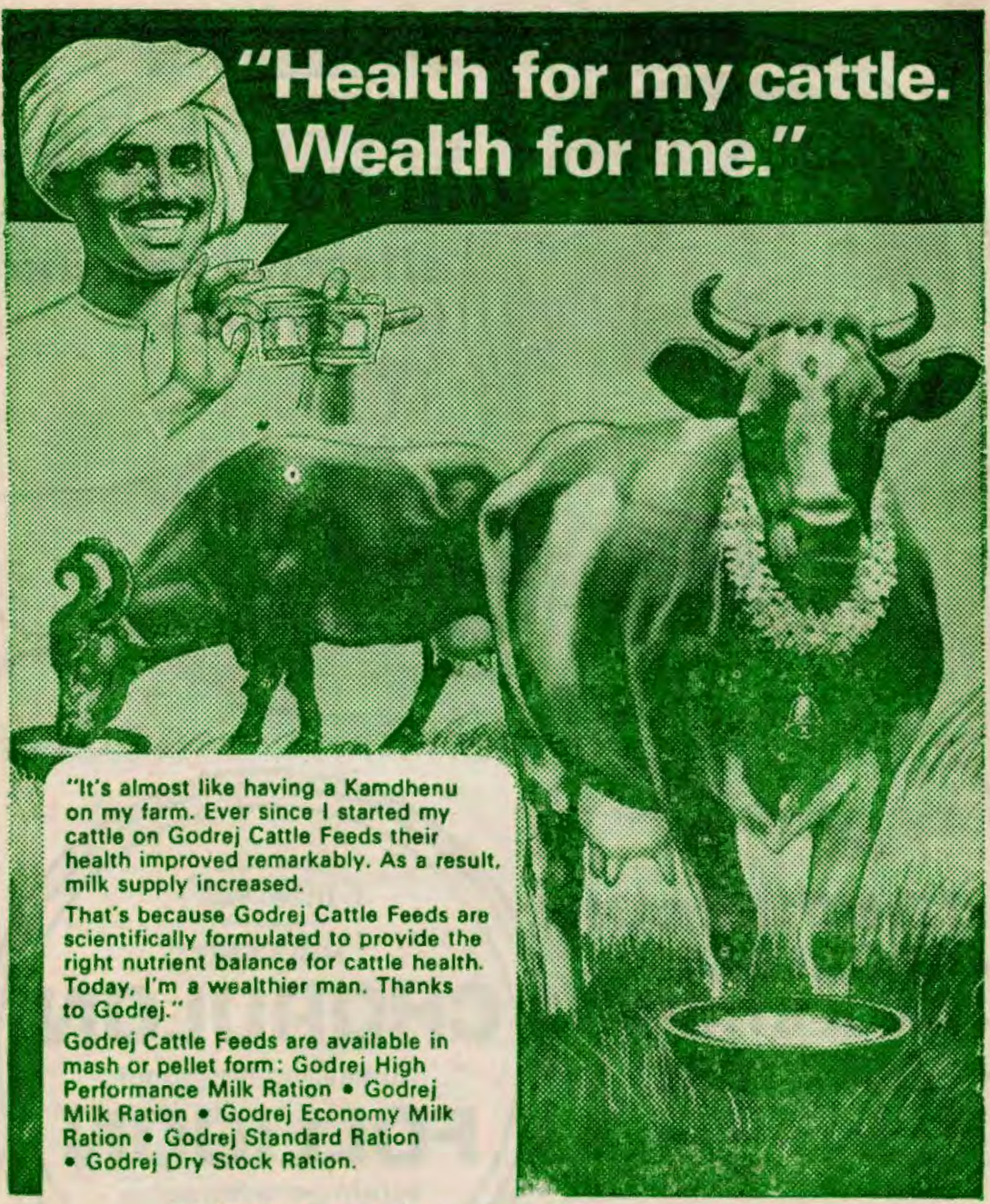
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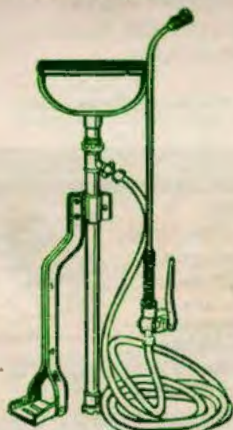
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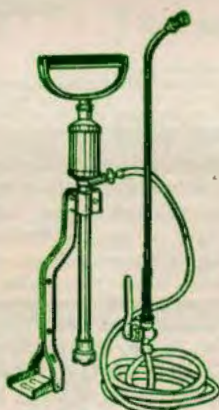
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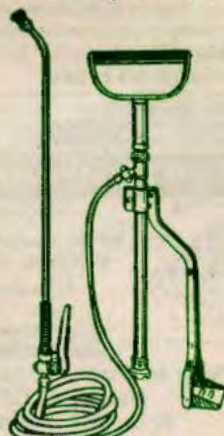
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3) ALOES COMPOUND as an Ovarian activator in

Anoestrus Buffaloes: (Dr. A.D. Deshpande, BSc. (Vet), I.C.D.P., Ahmedabad)

4) Effect of MYRON on Metritis in Cows & Buffaloes: (Dr. Gurmeet Singh, BVSc. & AH, Dr. Sushil Rattan, PVS., ADVS., Amritsar, Punjab.)

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It has dramatically changed the industrial skyline in Gujarat. Promoted by the Government of Gujarat and Gujarat State Fertilizers Company, Gujarat Narmada, with its 4.60 lakh shareholders, is Asia's largest company in respect of public shareholding. 1.30 lakh investors are farmers and their co-operatives. World renowned experts: Linde of West Germany, Texaco of USA, Haldor Topsoe of Denmark, BASF of West Germany and Snamprogetti of Italy have participated in its making: Based on a zero pollution concept, its electrostatic precipitators eliminate fly ash discharge, and a high stack of 125 metres, 1½ times taller than Qutab Minar, ensures highest dispersion factor of pollutants, if any.

Going on stream in December, 1981 and into commercial production in July, 1982, the plant has a massive production capacity of 1350 tonnes of Ammonia and 1800 tonnes of Urea per day. This goes to the farm sector in Gujarat,

Rajasthan, Madhya Pradesh, Punjab, Haryana and Uttar Pradesh. It helps in increasing food grain production by 28 lakh tonnes with an added income of Rs. 35 crores to the farmers.

Gujarat Narmada is already marketing its by product Liquid Nitrogen, Pure Sulphur recovered in its pollution control process, and surplus Ammonia, to industrial users. This results in a large saving of foreign exchange.

Gujarat Narmada plans ahead of today's needs, and many more projects are on the anvil.

Gujarat Narmada—a growth nucleus for the country.



**Gujarat Narmada Valley
Fertilizers Company Limited**

Registered Office: P.O. Narmadanagar, 392 015 Dist. Bharuch, Gujarat

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