

VOL. 4 No. 1 : AUGUST 1983



**The Indian  
Journal of  
Animal Reproduction**

JOURNAL OF THE  
INDIAN SOCIETY FOR THE  
STUDY OF  
ANIMAL REPRODUCTION

( Regd. No. Bom. 253/78 )

# THE INDIAN JOURNAL OF ANIMAL REPRODUCTION

Official Organ of the Indian Society  
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Vol. 4

No. 1

AUGUST 1983

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# Editorial

The Editorial Board feels happy to place in the hands of the readers the August 1983 issue of IJAR. Hope it will be appreciated that The Indian Journal of Animal Reproduction has certainly created an avenue for the scientists and field technicians to have their scientific papers on various aspects of Animal Reproduction published in the journal within a reasonable time. The journal certainly gets a credit for dissemination of the latest developments in the field of animal reproduction to a wider circle. It is our ambition that the journal should have a wider circulation and should reach all the Veterinary Institutions and Veterinary Hospitals in India.

Hope it will be appreciated that at present 75% of the Veterinarians are occupied in Livestock Development work in one way or the other; it will therefore be highly advisable that such a useful journal should be at their desk for providing the latest knowledge.

In this endeavour, it is necessary to have very close coordination with various agencies such as Agricultural Universities, State Animal Husbandry and Veterinary Departments, Research Institutions devoted to livestock development, Pharmaceuticals, Dairy Federations, Feed Factories, Stud farms, Goshalas and Panjarapoles to bear the burden of cost of production. Financial assistance in any form is welcome. The Editorial Board feels very grateful to ICAR New Delhi, for the financial assistance. Hope this example will be followed by allied institutions to promote the activities of ISSAR.

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## A Preliminary Report on Superovulation and Non-Surgical Embryo Collection in Cattle

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### ABSTRACT

The present investigation reports on the induction of superovulation in eleven cows with a regime involving intramuscular administration of PMSG and intrauterine infusion of PGF<sub>2</sub> alpha. A high degree of superovulation was obtained in 3 while a moderate rate was obtained in four and poor response in four cows. Four embryos and two unfertilized ova were successfully recovered by non surgical technique using two way Foley catheter from only one animal. The varying response with PMSG and the difficulties encountered during embryo collection are discussed.

\* \* \*

A multimillion dollar industry centered over superovulation, collection and transfer of embryo has evolved over the last decade, particularly in America, Canada and certain European countries. The major advantage of embryo transfer is as a tool for obtaining maximum number of offsprings from genetically superior and valuable cows. The technology is particularly useful in a country like India which has a large number of unproductive cattle and a few valuable cows. However, it is yet to take a foothold and the objective

of the present investigation, therefore, is to report the trials conducted on superovulation and embryo collection in cross-bred cows.

### Materials and Methods

The present investigation was carried out on 11 crossbred repeat breeder cows maintained at the dairy farm, HAU, Hissar. From the breeding and AI records, these animals were confirmed to be during day 10-14 of the oestrous cycle. Rectal examination was performed to approximately assess the stage of the oestrous cycle based on the stage of the development of the corpus luteum. These animals were subjected to a superovulation regime comprising of administration of 1500 I.U. of PMSG\* intramuscularly followed by intrauterine infusion of 5 mg of PGF<sub>2</sub> alpha (Lutelyse\*\*) forty eight hours later. All the animals were thereafter closely watched for oestrus symptoms and detection of the oestrus was facilitated by the use of a teaser bull. During oestrus which was observed 48 hours after PGF<sub>2</sub> alpha injection in all the animals, inseminations were performed thrice at twelve hours interval. Rectal and vaginal examinations were performed to assess the ovarian response to PMSG and PGF<sub>2</sub> alpha.

\* Antex Leo, Leo Pharmaceuticals Products, Denmark.

\*\* Lutelyse, Upjohn, U.S.A.



TABLE 1. OVARIAN RESPONSE TO PMSG AND PGF<sub>2</sub>  
ALPHA INJECTION

Cow No.	Day of PMSG injection	Day of PG injection	Ovarian response to day of injection		Remarks
			Right ovary	Left ovary	
1	12	14	12 CL	4 CL	Cervical impatency
2	17	—	Multiple follicles on both the ovaries		Anovulatory or lutenized follicles
3	11	13	10 CL	4 CL	Infusion done
4	12	14	3 CL	1 CL	— do —
5	14	16	Large multiple follicles on both ovaries		Anovulatory or lutenized follicles
6	13	15	1 CL	3 CL	Infusion done
7	12	14	1 CL	3 CL	Cervical impatency
8	10	12	1 CL	Smooth	Infusion not done
9	12	14	3 CL	1 CL	Infusion done
10	13	15	1 CL	—	Infusion not done
11	12	14	7 CL	8 CL	Infusion done

Response to PMSG—Based on corpora lutea

Excellent = 3 animals (> 10)

Moderate = 4 animals (2 to 4)

Poor = 4 animals (single or no ovulation)

CL = Corpora lutea

Attempts to recover the ova non-surgically was carried out on day 6 of the cycle. Initially, the superovulatory response was assessed by ovarian palpation. The method of non-surgical embryo collection in brief consisted of employing an epidural anaesthesia, followed by thorough cleansing of the perineal region of the animal. A two way Foley catheter was used for embryo collection. The air cuff placed at the base of one of the uterine horn was inflated with 15-20 ml of air. Fifty to sixty ml of enriched phosphate buffer saline (Betteridge, 1980) was introduced into the uterine horn and the same was withdrawn by aspiration with a syringe. The recovery of the flushing medium was facilitated by gently lifting the uterine horn. The horn was again flushed with another 50-60 ml of the flushing medium and procedure repeated 3-4 times. Simi-

larly, the other horn was flushed in fractions of 50-60 ml, 4-5 times. In all, about 500 ml of the flushing medium was used. The recovered medium was transferred into a measuring cylinder of 500 ml capacity and allowed to stand at room temperature; for about one hour. Thereafter, aliquots from the sediment were taken onto disposable petridishes and examined for the embryos under a phase contrast microscope.

## Results and Discussion

The pattern of response of the individual animals to superovulation regime is presented in Table I. It is evident that there were considerable variations in the degree of response between the individual animals. Out of eleven animals injected with PMSG, a high degree of



TABLE 2. DETAILS OF INFUSION DONE AND RECOVERY OF OVA

Cow No.	Amount infused (ml)	Amount recovered (ml)	% recovery (ml)	Type of fluid recovered	Remarks
3	270	200	75.9	Blood tinged	Scanning not attempted
4	470	400	85.1	Last 160 ml blood tinged	
6	550	460	83.6	Clear	No recovery ov ova
9	480	420	85.9	Clear	No recovery of ova
11	500	450	90.0	Clear	6 ova recovered

superovulation was obtained in only three wherein the number of corpora lutea were 15 or more. In four animals, superovulatory response was moderate wherein the number of corpora lutea were between 2-4. In the remaining four animals either failure of ovulation or single ovulation was observed. The variability in response to PMSG has been well documented. Factors such as individual variability, stage of the cycle, age, breed, nutritional plane and state of health have been frequently attributed to the variability in response to PMSG (Bellows and Short, 1972; Betteridge, 1977; Betteridge, 1980; Sugie *et al.*, 1980).

The single greatest advance in superovulation methodology in the last decade has been the use of PGF<sub>2</sub> alpha or its analogues. It has become routine now to inject PGF<sub>2</sub> alpha by intramuscular route about 48 hr after the gonadotropin treatment. However, the dose of PGF<sub>2</sub> alpha required for luteolysis is dependent upon the route of administration; a considerable lower dose is needed when administration is done through intrauterine route as compared to intramuscular or subcutaneous routes (Kaltenbach, 1980). In the present trial, all the animals infused with 5 mg of PGF<sub>2</sub> alpha intrauterine

were in oestrus starting from approximately 48 hrs. after its infusion.

Out of eleven animals subjected for superovulation, attempts to recover the embryo were made in only five animals. In the remaining six animals where collection was not attempted, two animals had impatency of the cervix, two animals had multiple follicles at the time of collection (probably lutenised) and another two had single ovulation each.

Out of the five animals where collection was attempted, a clear fluid in suitable proportion could not be obtained from two animals. The recovered fluid was generally blood tinged making the detection of embryos difficult. Additionally, these two animals were also the first from whom collection attempts were made. In the subsequent 3 animals, the technique was standardized and around 80-90% of the infused medium could be easily recovered (Table II).

Attempts to scan for the embryos were made in three. Ova could be successfully recovered in one of the animals. In the flushing medium obtained from the same animal, a total of 6 ova were recovered; two were unfertilized while four were fertilized compare. The rate of recovery of the ova compare favourably with that reported by Betteridge (1977).



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## Pineal Activity in Relation to Ovarian Structures and Breeding Seasons in the Water Buffaloes

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### ABSTRACT

Pineal glands from surthi buffaloes were analysed for weight, nucleic acids and protein. The data were analyzed for influence on ovarian functional status and season. Pineal weights and concentration of nucleic acid and protein were highest at follicular phase followed by luteal phase and smooth phase (no appreciable structures in the ovary) in both winter and summer. The increase in DNA content and decrease in RNA/DNA and protein/DNA ratio in summer suggest increased mitotic activity with decreased synthetic activity. The implications of altered pineal activity for poor breeding performance in summer is discussed in this paper.

\* \* \*

Pineal gland mediates most, if not all, of the effects of darkness on the endocrine (Nir, 1978) and reproductive system (Reiter, 1978). The photoperiodic differences between seasons alter pineal activity which in turn regulates reproductive activity in most species of animals (Reiter, 1980). Pineal is known to inhibit gonadal functions and this action is mediated by the hypothalamopituitary system (Wurtman, 1969; Wurtman, 1975; Fraschini and martini, 1970 and Mess et al., 1978). In surthi buffalo heifers the circ-

ulating levels of prolactin are high in summer (Sheth et al., 1978). This along with the low serum FSH to LH ratio (Janakiraman, et al., 1980) is believed to be responsible for the poor reproductive performance of these animals in Summer. Because of the role of pineal in regulating PRL (Fisher and Lapwood, 1981) and gonadotrophins (Fraschini et al., 1968), we were interested to study the pineal activity in surthi buffaloes in relation to ovarian function. This paper deals with the changes in pineal weight, Nucleic acids and protein content in winter and summer in relation to ovarian structures.

### Materials and Methods

The pineal glands were collected in winter and summer, from surthi buffaloes or heifers slaughtered between 5 and 6 a.m. The gland was removed within 15-30 minutes of killing the animal and were brought to the laboratory in ice cold condition. The tissue after cleaning and weighing were preserved at -20°C until analyzed. The pineal glands thus collected were divided into three groups according to ovarian structures i.e., Smooth phase (No appreciable structures on the ovary), follicular phase (10 mm or larger follicle (s) on the ovary) and luteal phase (10 mm or larger active CL). 10% homogenate in 0.9% KCL was made from single or pooled pineal tissues and DNA and



RNA (Volkin and Kohn, 1964) and protein (Gormall et al., 1949) were estimated. The data were analyzed using Analysis of variance (Steel and Torrie, 1964).

## Results and Discussion

76 pineal glands were collected in winter (November-February) and 74 in summer (April-July). The collection was random and the Table-1 gives fresh weight of pineals and the number of animals with different ovarian structural/functional status. The number of animals with no structures in the ovaries (Smooth) were greater in summer than in winter months (39 against 8) there were no

TABLE 1. FRESH WEIGHT OF THE PINEAL GLANDS IN THE SURTI BUFFALO

Season	Structure of the Ovary		
	Smooth	Follicular	Luteal
Winter	189 <sup>(8)</sup>	164 <sup>(40)</sup>	158 <sup>(28)</sup>
	$\pm 46.5$	$\pm 7.7$	$\pm 11.4$
Summer	171 <sup>(39)</sup>	187 <sup>(18)</sup>	175 <sup>(17)</sup>
	$\pm 11.5$	$\pm 20.3$	$\pm 22.3$

Values in Parentheses indicate number of animals. weight (mg)

TABLE 2. NUCLEIC ACIDS AND PROTEIN IN THE PINEAL GLANDS OF SURTI BUFFALO

	Season	
	Winter (27) <sup>n</sup>	Summer (49) <sup>n</sup>
DNA mg/g	$3.96 \pm 0.24$	$5.57^{*} \pm 0.26$
RNA mg/g	$2.13 \pm 0.10^{(0.54)}$	$2.39^{*} \pm 0.10^{(0.43)}$
PROTEIN g%	$86.5 \pm 2.8^{(21.8)}$	$89.7 \pm 3.8^{(16.1)}$

(n) = Number of observations. Values given in Parentheses are ratios of RNA and Protein to DNA.

\*  $P < 0.05$ ; \*\*  $P < 0.01$

significant differences in fresh weight of pineal glands either between seasons or between different phases within a season. However, the weights tended to be higher in summer.

The data on DNA, RNA and Protein in the pineals were examined for seasonal differences after pooling the values in different phases (Table-2). Pineal concentration of DNA was higher in summer there being no differences in the RNA or protein levels.

The increased DNA concentration is suggestive of higher mitotic activity or duplication of chromosomes in preparation to that. Earlier histological studies (Unpublished data) have shown that the proportion of pinealocytes to glial cells significantly increased in summer in pineals collected from surthi buffalo heifers. The absence of corresponding increase in RNA and protein in summer indicates that increased mitotic activity is not accompanied by synthetic activity. The data on pineal constituents were analyzed for differences between different phases of ovarian activity pooling the values from both seasons (Table-3). Highest levels of nucleic acids and protein were present in the follicular phase followed by luteal phase and smooth phase. The ovarian hormones have been reported to inhibit pineal activity (wurtman et al; 1968) in rat. In the buffalo it appears that the pineal is free from such inhibitory action.

The influence of breeding seasons on the pineal gland composition in different phases of ovarian activity was examined by analysis of variance (Table-4 and 4a). Both in peak breeding winter and low breeding summer follicular phase coincided with highest pineal concentration



TABLE 3. NUCLEIC ACIDS AND PROTEIN IN THE PINEAL GLANDS OF SURTI BUFFALOES AS PER OVARIAN STRUCTURE

	Smooth	Follicle	Corpus luteum
DNA	3.97	5.71	5.11
mg/g	$\pm 0.20$	$\pm 0.40$	$\pm 0.44$
RNA	2.15	2.45	2.13
mg/g	$\pm 0.10$	$\pm 0.10$	$\pm 0.14$
PROTEIN	68.6	105.4	89.3
g%	$\pm 1.9$	$\pm 4.3$	$\pm 4.4$
RNA/DNA	0.54	0.43	0.42
PROTEIN/DNA	17.3	18.4	17.5

Values are means  $\pm$  SE for 22 observations.

TABLE 3A. ANALYSIS OF VARIANCE FOR PINEAL NUCLEIC ACIDS AND PROTEIN

Source	df	DNA		RNA		PROTEIN	
		mss	F	mss	F	mss	F
Phase	2	17.18	5.99**	0.715	2.15	7457.70	24.49**
Error	63	2.87	—	0.333	—	304.46	—

\*\* P < 0.01

TABLE 4. NUCLEIC ACIDS AND PROTEIN IN THE PINEAL GLANDS OF SURTI BUFFALOES

	Ovarian structure	Seasons	
		Winter	Summer
DNA	Smooth	$4.44 \pm 0.44$	$4.08 \pm 0.26$
mg/g	Follicle	$4.36 \pm 0.10$	$7.21 \pm 0.45$
	Corpus luteum	$2.85 \pm 0.13$	$6.40 \pm 0.23$
RNA	Smooth	$2.00 \pm 0.06$	$2.18 \pm 0.14$
mg/g	Follicle	$2.34 \pm 0.02$	$2.79 \pm 0.08$
	Corpus luteum	$1.73 \pm 0.27$	$2.31 \pm 0.19$
PROTEIN	Smooth	$73.1 \pm 6.2$	$69.7 \pm 2.3$
g%	Follicle	$95.1 \pm 3.8$	$121.4 \pm 9.1$
	Corpus luteum	$83.6 \pm 4.5$	$92.6 \pm 6.5$

TABLE 4A. ANALYSIS OF VARIANCE FOR PINEAL NUCLEIC ACIDS AND PROTEIN

Source	df	DNA		RNA		Protein	
		mss	F	mss	F	mss	F
Season	1	46.82	9.78**	1.25	1.05	182.4	0.05
Phase	2	13.41	2.80	1.33	1.12	7480.9	2.22
Season $\times$ Phase	2	4.78	1.86	1.19	3.72	3370.3	14.45**
Error	70	2.50	—	0.92	—	293.3	—

\*\* P < 0.01



of nucleic acids and protein. In low breeding summer luteal phase witnessed higher levels of nucleic acids and protein compared to smooth phase. There were no seasonal differences when the ovaries were without active structures.

The fact that the concentration of nucleic acids and protein were higher when the ovaries had active structures (follicle or corpus luteum) than not, indicate that a relationship exists between the functional status of these two organs. The elucidation of the above relationship warrants further investigations.

Increased level of circulating prolactin could be one of the factors involved in poor development of the follicles and for inhibition of ovulation in case of developed follicles. This invariably occurs

in summer months. Fisher and Lapwood (1981) in their studies on lambs have clearly established that seasonal differences in prolactin levels is mediated by the photoperiodic influences on the pineal gland. Thus it is possible that in the buffalo too, higher prolactin levels in summer are caused by seasonal changes in pineal metabolism. In turn the higher levels of prolactin may make the ovaries refractory to the influence of FSH and LH. This leads to anovulatory estrous cycles and consequent poor breeding performance in summer.

### Acknowledgement

This forms part of research the work under the All India Co-ordinated research project on Buffalo-Anand Centre.

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## Estradiol-17B Levels During Early Pregnancy in Buffaloes

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### ABSTRACT

Peripheral blood samples were collected from twelve buffalo heifers on days 0,1,3,5,7,9,11,13,15 to 22 following breeding to monitor the changes in estradiol-17B levels during estrus, early luteal, mid luteal, late luteal and post luteal phases. The estradiol-17B levels were higher and significant ( $P < 0.01$ ) on the day of estrus from 19 days of insemination in non pregnant and from 22 days of insemination in pregnant buffalo heifers. The fertile buffaloes had a slightly higher concentration on the preovulatory day (day 0) and on ovulatory day (day 1). The analysis of variance revealed a very significant difference ( $P < 0.001$ ) between the days of breeding both in pregnant and non pregnant buffaloes. However, the overall mean levels between pregnant and non pregnant were not significantly different upto 22 days of breeding.

\* \* \*

Sufficient information on the estimations of estradiol-17B during estrus and pregnancy is available for cows. The changes in the levels of estradiol-17B during estrus and early pregnancy are not adequately studied in buffaloes. Therefore the present studies were undertaken to study the circulatory levels of estradiol-17B in fertile and nonfertile buffalo heifers following breeding.

### Materials and Methods

The experimental 12 Murrah buffaloes

(nulliparous) were selected from the herd of National Dairy Research Institute, Karnal and all were kept under the similar managerial and feeding conditions. Buffaloes were moved into a nearby unneated stall prior to each sampling. The blood was collected from the jugular vein before feeding each day between 0800 and 1000 hr on days 1,3,5,7,9,11,13,15,16,17,18,19,20,21 and 22 except on day 0 where blood sampling was done in the morning hours ( $d_{01}$ ) and evening hours ( $d_{02}$ ) of post insemination. The samples were kept in ice immediately after collection and the plasma was separated by centrifuging at 3000 rpm for 30 minutes and stored in different aliquots at  $-15^{\circ}\text{C}$  pending luteinizing and estradiol-17B hormone analysis.

Estrus was detected by employing a combination of aids. A vasectomized buffalo bull was paraded in the shed where buffalo heifers were kept. Buffaloes mounted upon by the bull were considered to be in estrus. Buffaloes were also checked for their temperament and mounting behaviour (standing heat) on other buffaloes. They were regularly checked for the vaginal congestion, swollen vulva, micturition and vaginal mucus discharge. The genitalia of these buffaloes were examined per rectum for the tone of the uterine horns and for the presence of mature follicles to confirm the stage of estrus and ovulation. All buffaloes were inseminated twice (morning and evening) and pregnancy was con-



TABLE 1. DIRECT EVALUATION OF ESTRADIOL-17B  
RADIOIMMUNOASSAY FOR ACCURACY

No. of Replicates	Estradiol-17B (pg) added	Estradiol-17B (pg) recovered	C.V.	Overall statistical values
4	16	17.28 ± 1.18	11.85	r = 0.9975 (P < 0.01)
8	32	31.16 ± 0.63	3.50	b = 1.0855
4	64	68.69 ± 1.31	3.30	y = 0.3065

± = SEM; C.V. = Coefficient of variation  
r = Coefficient of correlation;  
b = regression and y = dependent variable.

firmed by rectal palpation at 60 days after insemination.

#### Radioimmunoassay of estradiol-17B:

The estradiol-17B levels in the peripheral blood plasma were measured (Jain and Pandey, 1981) with some modification. The sensitivity of the radioimmunoassay for estradiol-17B estimation was one pg per tube and the coefficient of variations between and within assays were 6.42 and 4.80, respectively. The specificity for estradiol-17B antiserum was reported earlier (Korenman *et al.*, 1974) whereas the accuracy is given in Table 1.

#### Statistical Analysis:

The least square technique (Harvey, 1979) was used to study the variations between collection intervals during estrus and early pregnancy. The significant difference in the least square means for each effect was studied (Duncan, 1955).

### Results and Discussion

*Estradiol-17B levels* (Table 2): The estradiol-17B concentration during the day of estrus (day 0) was found to be very high (25.37 ± 8.9 pg/ml) on first 12 hr of estrus and 26.24 ± 3.34 pg/ml between 12-24 hr of estrus. The levels started

TABLE 2. MEAN PLASMA LEVELS OF ESTRADIOL-17B (pg/ml) IN PREGNANT AND NON PREGNANT BUFFALO HEIFERS AFTER BREEDING

Days of insemination	Pregnant Mean SEM N	Nonpregnant Mean SEM N
0 <sub>1</sub>	32.40 ± 6.87(5)	25.37 ± 8.90(7)
0 <sub>2</sub>	40.03 ± 13.84(5)	26.24 ± 3.34(7)
1	10.81 <sup>ab</sup> ± 4.67(5)	13.06 ± 4.81(7)
3	7.84 <sup>ab</sup> ± 1.87(5)	7.74 <sup>cd</sup> ± 1.49(7)
5	12.50 <sup>ab</sup> ± 1.90(3)	7.18 <sup>cd</sup> ± 2.23(7)
7	6.85 <sup>ab</sup> ± 2.41(4)	6.01 <sup>bc</sup> ± 2.34(7)
9	6.08 <sup>ab</sup> ± 1.99(5)	8.63 <sup>cd</sup> ± 3.08(7)
11	8.58 <sup>ab</sup> ± 2.28(5)	5.84 <sup>cd</sup> ± 2.12(6)
13	9.79 <sup>ab</sup> ± 1.51(5)	12.74 ± 3.20(6)
15	6.50 <sup>ab</sup> ± 1.78(5)	12.74 ± 3.37(6)
16	11.47 <sup>ab</sup> ± 2.70(3)	5.70 <sup>cd</sup> ± 2.25(6)
17	6.40 <sup>ab</sup> ± 2.28(5)	9.58 ± 3.22(6)
18	8.30 <sup>ab</sup> ± 2.00(5)	11.78 ± 3.19(6)
19	8.60 <sup>ab</sup> ± 2.54(5)	3.33 <sup>cd</sup> ± 0.79(5)
20	5.16 <sup>ab</sup> ± 1.06(5)	11.47 ± 3.46(5)
21	6.87 <sup>ab</sup> ± 2.94(4)	20.67 ± 5.17(5)
22	10.71 <sup>ab</sup> ± 2.25(4)	20.71 ± 3.82(5)

a different from 32.40; b different from 40.03;  
c different from 25.37 and d different from 26.24  
(P < 0.01)



declining from days 1 to 11 after insemination in non pregnant buffalo heifers. A minor increase was observed on days 13, 15 and 18 and a major increase on days 21 and 22 of the cycle. A highly significant difference ( $P < 0.01$ ) in estradiol-17B levels on days 3, 5, 7, 9, 11, 16 and 19 and ( $P < 0.05$ ) on days 1, 17, 18 and 20 from the levels of 25.37 pg/ml was observed during the first 12 hr of the commencement of estrus symptoms. The estradiol-17B levels during the period of 22 days of breeding in heifers found to be pregnant did not fluctuate much and no fall in estradiol-17B level were noticed after day 21. The basal levels of  $5.16 \pm 1.06$  to  $0.79 \pm 1.52$  pg/ml were observed during all the days except on day 0 with a peak of  $32.40 \pm 6.87$  and  $40.03 \pm 13.84$  pg/ml at a collection interval of 12h and minor peaks of  $10.81 \pm 4.67$ ,  $12.50 \pm 1.90$ ,  $11.47 \pm 2.70$  and  $10.71 \pm 2.25$  pg/ml on day 1, 5, 16 and 22 of insemination. Analysis variance showed a significant difference ( $P < 0.01$ ) between days of insemination (Table 3). However, the overall mean levels of estradiol-17B between pregnant and non-pregnant buffaloes did not differ significantly (Table 3).

TABLE 3. ANALYSIS OF VARIANCE  
BETWEEN PREGNANT AND NON  
PREGNANT BUFFALO HEIFERS  
FOR ESTRADIOL-17B LEVELS

Source of variation	d.f.	m.s.s.
Between pregnant and nonpregnant	1	10.13
Error	180	141.61
Total	181	—
Between days (Non-pregnant)	16	306.5454***
Error	87	95.0398
Total	103	—
Between days (Pregnant)	16	452.6881***
Error	61	101.6986
Total	77	—

\*\*\* Significant ( $P < 0.001$ )

The peak levels of estradiol-17B concentration found in the present investigation during estrus were similar (Dobson and Dean, 1974; Monk *et al.*, 1975), higher (Wettemann and Hafer, 1973; Smith *et al.*, 1975) and lower (Shemesh *et al.*, 1972; Arije *et al.*, 1974) in cows. These levels are also lower to (Kamonpatana *et al.*, 1979) and higher to (Bachleas *et al.*, 1979) in buffaloes. It is believed that maximum secretion of estradiol-17B occurred from the preovulatory follicles around the onset of estrus (Moore *et al.*, 1969; McCracker *et al.*, 1971; Bird, 1978). The variability in the secretion of estradiol-17B observed in the present study is comparable with the random development of follicles throughout the late luteal phase and also probably reflects the short term fluctuations in the secretions of steroids from the same follicle (Boird, 1978). Jain and Pandey (1983) reported an increase in the secretion of estradiol-17B within the hours of each pulse of LH, indicating that the follicles in buffaloes were extremely sensitive to comparatively minor fluctuations in the LH concentration and reflected by a positive coefficient of correlation between LH and estradiol-17B on all days and hours of blood collection ( $r = 0.2294$  to  $r = 10.9999$ ). It was possible that the pulses represent oscillations of the negative feed back loop involving estradiol-17B from the follicles and LH from the adenohypophysis.

The subsequent minor peaks of estradiol-17B noted on days 13 and 15 in non-pregnant and on day 16 in pregnant buffalo heifers were similar to (Varman *et al.*, 1964) in bovine heifers. The levels observed during early luteal and late luteal periods were also similar (Batson *et al.*, 1974) for bovine heifers.



### Acknowledgement

The authors feel grateful to Dr. G.D. Niswender, Colorado State University

Fort Collins for providing the specific antisera to estradiol-17B to carry out this study.

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## Genital Abnormalities in Indigenous and Exotic Female Pigs : Prevalence, Gross and Microscopic Appearance

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Swine, being a prolific breeder is considered as one of the farm animals having high potentiality for greater economic gains. Production is directly dependent on the efficiency of reproduction which is modified by genital abnormalities. Hence there appears a great need for detail studies on the genital abnormalities of pig in order to have a better understanding on their prevalence, type and nature which in turn will help in evolving suitable measures to combat these. Most of the studies conducted so far on genital abnormalities pertained to exotic pigs of the developed countries and emphasized much on recording the incidence of gross abnormalities (Wiggins *et al.*, 1950; Nalbandov, 1952; Herman and Vandeplassche, 1969; Einarsson and Gustafsson, 1970; Reed, 1970; Cherkasova and Danilko, 1972; and Karlberg, 1979). Reports on genital abnormalities in indigenous pigs are very limited in the available literature (Nath, 1976 and Viswanath *et al.*, 1979). The study reported herein, therefore, has been undertaken to record the prevalence and details of gross and microscopic appearance of the genital abnormalities in indigenous and exotic pigs of Assam.

### Materials and Methods

A total of 120 female genital organ of

pigs collected from the local slaughter houses and the All India Co-ordinate Research Project on pigs, College of Veterinary Science, Khanapara constituted the material for the study. A total of 82 genital organ from indigenous and 38 from exotic could be obtained. Immediately after slaughter the genital organs were removed from the carcass and carried in sterile polythene bags to the laboratory for examination. All parts of each organ were thoroughly examined for gross pathological lesions within one hour of collection and were recorded.

Representative pieces of tissue from the vagina, cervix, uterus, Fallopian tubes and ovaries of each of the genital organs were collected and preserved in 10 per cent formal-saline solution. After 48 to 72 hours of fixation the tissue samples were processed by commonly used procedure and finally embedded in paraffin. Tissue sections of 4 to 6 microns in thickness were prepared and stained with haematoxylin and eosin as per routine procedure. The tissue sections were then examined and microscopic changes were recorded in detail. The incidence of ovarian and tubular abnormalities was found out separately for indigenous and exotic pigs.

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TABLE 1. PREVALENCE OF OVARIAN AND TUBULAR ABNORMALITIES IN THE FEMALE PIG.

Abnormality	Prevalence (%)	
	Indigenous (82)	Exotic (38)
Ovarian	8.54 (7)	13.16 (5)
Tubular	7.32 (6)	10.53 (4)
Mixed ovarian & tubular	18.29(15)	23.68 (9)
Total	34.15(28)	47.37(18)

Figures in the parentheses indicate number of observation.

## Results and Discussion

Out of 82 genital organs from indigenous and 38 from exotic pigs 34.15 and 47.37 per cent respectively revealed gross pathological lesions. The incidence of ovarian, tubal and mixed ovarian and tubal abnormalities recorded as 8.54, 7.32 and 18.29 per cent respectively in indigenous was slightly lower than the respective figures of 13.16, 10.53 and 23.68 per cent recorded in exotic pigs (Table 1).

### Ovarian abnormalities:

Prevalence of different types of ovarian abnormalities has been shown in table 2. Existence of these ovarian abnormalities

in the pig has been well documented in the literature (Wiggins *et al.*, 1950; Reed, 1970; Einarsson, 1970; Silobad, 1971; Nath, 1976 and Viswanath *et al.*, 1979) except the condition diagnosed as haemosiderosis of the ovary which occurred in the frequencies of 3.66 and 2.63 per cent in indigenous and exotic pigs respectively. Amongst all types cystic ovary, paraovarian cyst and oophoritis were found to be commonly occurring ovarian abnormalitie in both indigenous and exotic pigs. Earlier reports indicated that the incidence of cystic ovary varied in a wider range. Nalbandov, (1952), Thain (1965) and Vandeplasseche *et al.*, (1971) recorded the incidence of cystic ovary

TABLE 2. NATURE OF INVOLVEMENT AND PREVALENCE OF DIFFERENT TYPES OF OVARIAN ABNORMALITIES IN INDIGENOUS AND EXOTIC PIGS

Abnormalities	Number involved		Total No observed				Prevalence (%)	
	Bilateral Unilateral		I	E	I	E	I	E
	I	E						
Single cyst	—	1	1	1	1	2	1.22	5.26
Multiple small cyst	3	4	—	—	3	4	3.66	10.53
Multiple large cyst	—	1	—	—	—	1	0.00	2.63
Cystic corpus luteum	3	1	—	—	3	1	3.66	2.63
Paraovarian cyst	13	12	—	—	13	12	15.85	31.58
Ovaro-bursal adhesion	4	2	—	—	4	2	4.88	5.26
Oophoritis	12	4	—	—	12	4	14.63	10.53
Intra-ovarian haemorrhage	2	2	1	—	3	2	3.66	5.26
Haemosiderosis	1	—	2	1	3	1	3.66	2.63

I: Indigenous, E: Exotic



varying from 10 to 39 per cent. Einarsson and Gustafsson (1970), Silobad (1971) and Viswanath *et al.*, (1979) observed paraovarian cyst in 3 to 23 per cent of the genital organs. The incidence of oophoritis recorded in the present study, (10.53 to 14.63%) however, was similar to that reported by Silobad (1971).

Details of gross and microscopic appearance of different ovarian abnormalities have been shown in table 3. It was observed that small cyst in the ovaries did not reveal appreciable pathological changes except in that both cystic follicle and cystic

corpora lutea were larger than the normal structures in size. However, atrophy of the ovarian stroma was the important pathological change observed in the single case of multiple large cysts in exotic pigs. Oophoritis, intra-ovarian haemorrhage and haemosiderosis were apparent on microscopic examination only.

#### *Tubular abnormalities:*

Prevalence of different tubular abnormalities in pig genital organs is shown in table 4. Endometritis, papillary hyperplasia, cervicitis and vaginitis were the

TABLE 3. GROSS AND MICROSCOPIC APPEARANCE OF OVARIAN ABNORMALITIES IN FEMALE PIGS

Abnormalities	Gross appearance	Microscopic appearance
Single cyst	1-2.2 cm in diameter, some cysts luteinized fluid amber coloured.	Non luteinized resembles follicle, luteinized resembles corpus luteum.
Multiple small cyst	1-1.5 cm in diameter, fluid clear watery	Resembles mature follicle
Multiple large cyst	Cysts 3-4.5 cm in diameter,	Cystic wall of connective tissue and lined by flat cells, ovarian stroma atrophied.
Cystic CL	Large irregular central cavity filled with amber coloured fluid	Cavity lined by loose connective tissue, lutein cells vacuolated.
Paraovarian cyst	0.3-3.5 cm in diameter, appears greyish and translucent	Cystic wall lined by low cuboidal cells surrounded by thick fibrous tissue, eosinophilic substance
Ovaro-bursal adhesion	Ovaries encapsulated by fibrous strands in severe cases	—
Oophoritis	Not apparent	Lymphocytic, eosinophilic and erythrocytic infiltration beneath tunica albuginea, fibrous proliferation in chronic cases.
Intra-ovarian haemorrhage	Not apparent	Congestion, erythrocytic infiltration with few eosinophils beneath the tunica albuginea
Haemosiderosis	Not apparent	Presence of haemosiderin pigments and free erythrocyte beneath tunica albuginea.



TABLE 5. GROSS AND MICROSCOPIC APPEARANCE OF DIFFERENT TUBULAR ABNORMALITIES IN FEMALE PIGS.

Abnormalities	Gross appearance	Microscopic appearance
Hydrosalpinx	Tubular wall thinner and semi-transparent containing fluid, adhesion present	Atrophied epithelium, mild mononuclear cellular infiltration
Fibroma molle (infundibulum)	Presence of nodules of 0.3-0.6 cm in diameter with illdefined neck in inner infundibular region, enlargement and adhesion of the infundibulum	Nodules contain mucinous material, interlacing bundles of fibroblast and collagen fibres, fusiform neoplastic cells, large, oval or elongated nuclei, tumour cells demarcated by capsule.
Pyosalpinx	Distended ampulla containing semiliquid, greyish white material	Polymorphonuclear, eosinophilic and less frequently plasma cell infiltration into mucosa and submucosa
Cystic endometrium	Horns elongated, soft and thick walled, mucosa glistening, presence of cystic elevations of about 1 cm diameter in the mucosa	Uterine glands near the cysts atrophied, proteinous exudate, occasionally lymphocytic and eosinophilic infiltration
Segmental aplasia	Left horn and fallopian tube in one, part of left horn in the other and both infundibulae in the third were missing, hydrosalpinx and adhesion were also present	
Granular vulvo-vaginitis	Congested nodules in the mucosa of vulva and posterior part of the vagina	Nodules formed by the lymphocytic aggregation in the submucosal area, congestion
Persistent hymen	A dorso-ventral band of tissue just anterior to the urinary meatus	
Subserous cyst (body & tube)	Cysts 0.4 to 0.5 cm in diameter containing watery fluid present beneath the serosa	Cystic wall made of connective tissue lined by flat epithelial cells
Salpingitis	Not apparent except whitish serous fluid in the lumen	Infiltration of mononuclear cells with erythrocytes and occasionally eosinophils to mucosa and submucosa. Denudation of mucosa and connective tissue proliferation in chronic cases.
Endometritis	Congestion of the mucosa, presence of blood stained mucus in the lumen, fibrous strands on the body in few	Congestion and cellular infiltration to mucosa & submucosa. Desquamation of the epithelium, connective tissue proliferation and plasma cell infiltration in chronic cases.



TABLE 5 CONTD.

Abnormalities	Gross appearance	Microscopic appearance
Papillary hyperplasia (tube, body, vagina)	Not apparent	Multilayered epithelium, marked increase in the papillary projections, lymphocytic and erythrocytic infiltration in the lamina propria.
Haemosiderosis (uterus)	Not apparent	Deposition of haemosiderin pigment and presence of free erythrocytes within the endometrium.
Melanosis (body)	A small black patch on the mucosa	Deposition of melanin pigment in the endometrium, surrounding blood vessels are congested.
Metaplastic changes (tube)	Not apparent	Metaplasia of columnar to squamous epithelium, inflammatory changes present.
Cervicitis	Oedema and congestion of the mucosa, cervix hard in some cases	Congestion, infiltration of lymphocytes, erythrocytes and occasionally eosinophils in the mucosa and submucosa. Fibrous proliferation and plasma cell infiltration in chronic cases.
Vaginitis	Oedema and congestion of mucosa, presence of mucus in the lumen	Mild epithelial hyperplasia, infiltration of lymphocytes and occasionally polymorphonuclear and plasma cells.

fallopian tube of indigenous pig was similar to that of fibroma of horse and other animals as described by Stannard and Pulley (1978). Inflammatory conditions such as salpingitis, cervicitis, endometritis and vaginitis revealed typical inflammatory changes like cellular infiltration and congestion on microscopic examination. Connective tissue proliferation was the commonly occurring microscopic change in salpingitis, endometritis and cervicitis in chronic cases. Denudation of the epithelium was recorded in salpingitis and endometritis only.

### Summary

Out of 82 indigenous and 38 exotic genital organs respectively 34.15 and

47.37 per cent revealed abnormalities. The incidence of ovarian, tubular and mixed ovarian and tubular abnormalities was 8.54, 7.32 and 18.29 per cent respectively in indigenous and 13.16, 10.53 and 23.68 per cent respectively in exotic pigs. Ovarian abnormalities were single cyst, multiple small cyst, multiple large cyst, cystic corpora lutea, para-ovarian cyst, ovaro-bursal adhesion, oophoritis, intra-ovarian haemorrhage and haemosiderosis in the frequencies of 1.22, 3.66, 0.00, 3.66, 15.85, 4.88, 14.63, 3.66 and 3.66 per cent respectively in indigenous and 5.26, 10.53, 2.63, 2.63, 31.58, 5.26, 10.53, 5.26 and 2.63 per cent respectively in the exotic. Small ovarian cysts did not reveal pathological change



on microscopic examination while atrophy, of the ovarian stroma was the important pathological change observed in multiple large cysts. Oophoritis, intra-ovarian haemorrhage and haemosiderosis were apparent on microscopic examination only. Abnormalities involving the tubular part were hydrosalpinx, fibroma molle, pyosalpinx, cystic endometrium, segmental aplasia, granular vulvo-vaginitis, persistent hymen, subserous cyst, salpingitis, endometritis, papillary hyperplasia, haemosiderosis, melanosis, metaplastic changes, cervicitis and vaginitis in the respective frequencies of 2.44, 1.22, 0.00, 6.10,

2.44, 4.88, 2.44, 2.44, 12.20, 20.73, 13.41, 2.44, 0.00, 6.10, 14.63, and 18.29 per cent in indigenous and 0.00, 0.00, 2.63, 7.89, 2.63, 5.26, 13.16, 0.00, 7.89, 15.79, 10.52, 0.00, 2.63, 7.89, 13.16 and 23.68 per cent in exotic. Papillary hyperplasia, haemosiderosis and metaplastic changes of the epithelium were apparent on microscopic examination only and were associated with inflammatory changes. Inflammatory changes were typical in salpingitis, cervicitis, endometritis and vaginitis. Denudation of the mucous membrane was observed in salpingitis and endometritis.

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## Oestrus and its Relation to Conception in Rural Cattle

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### ABSTRACT

A study was undertaken on the time of commencement and stage of oestrus in 115 rural cattle and their relationship with conception. In more than 80.00% of the rural cattle, oestrus commenced during the cool hours (i.e. morning and evening hours) and it was also found that cool hours favoured the occurrence of conception. The stages of oestrus in the animals that came for inseminations could be grouped into early, mid and late heat and these were 11.30%, 57.39% and 31.30% respectively. The highest percentage of animals (54.00%) became pregnant when inseminated in mid-heat. Further, maximum conception (51.61%) resulted in animals showing typical fern-pattern in cervical mucus.

\* \* \*

A study was made on oestrous behaviour and its relation to conception in rural cattle. The findings are reported here.

### Materials and Methods

The present study was conducted on the Cattle belonging to the adopted villages of operational Research Project Rithoura (I.V.R.I., Izatnagar). Observations were recorded on the time of commencement of oestrus and stage of oestrus in 115 animals and their relationship with the pregnancy was assessed by pregnancy check-up after inseminating these animals. The classification of the

time of commencement of oestrus was done as follows:

- I. 4.00 A.M. to 10.00 A.M. (Morning hours).
- II. 10.00 A.M. to 4.00 P.M. (Early noon to afternoon hours).
- III. 4.00 P.M. to 10.00 P.M. (Early evening to late evening hours).
- IV. 10.00 P.M. to 4.00 A.M. (Night hours).

The stage of oestrus was classified as early, mid and late heat on the basis of the history regarding the time of commencement of heat as spelled out by the farmers and assessed on the findings after per rectum examination of the genital organs and vaginal inspection.

The fern-pattern was categorised as typical, atypical and no-pattern (Luktuke and Roy, 1967). The fern-pattern of cervical mucus during oestrus was tested in relation to pregnancy in 50 animals.

### Results and Discussion

#### A. Time of commencement of oestrus:

In the present study it was noted that 56.67% of the heifers and 34.12% of the cows were observed in heat by the farmers during morning hours while 26.67% of heifers and 51.16% cows in early to late evening hours and only 16.67% of the heifers and 14.12% of the cows were noticed in heat by the farmers during early noon to afternoon hours. However



TABLE 1. STAGE OF OESTRUS IN RELATION TO PREGNANCY.

Animal type	No. exam.	Animals diagnosed in			No. followed for pregnancy			Pregnant diagnosed		
		Early heat (A)	Mid heat (B)	Late heat (C)	A	B	C	Early heat (A)	Mid heat (B)	Late heat (C)
Heifer	28	6 (21.43)	10 (35.71)	12 (42.86)	4	10	8	1 (25.00)	6 (60.00)	2 (25.00)
Cow	87	7 (8.04)	56 (64.37)	24 (27.59)	5	40	20	2 (40.00)	21 (52.50)	4 (20.00)
Total	115	13 (11.30)	66 (57.39)	36 (31.30)	9	50	28	3 (33.33)	27 (54.00)	6 (21.43)

Figures in parenthesis indicate percentage.

none of the animal was reported by the farmers in heat during night hours (i.e. between 10.00 P.M. to 4.00 A.M.) indicating that the farmers did not observe oestrus symptoms during night hours. Thus it is apparent that in about 80 to 85% of the rural cattle, the oestrus symptoms commenced during cool hours (i.e. morning and evening hours). Maximum percentage of oestrus occur during cool hours (Ahuja *et al.*, 1961; Chaudhary *et al.*, 1965; Sharma *et al.*, 1968 and Purbey and Sane 1978) in the cattle maintained at organised farms. This indicated that the ambient temperature played some role in the commencement of oestrus. 31.43% and 52.08% of the animals that were in heat during morning and early to late evening hours respectively, when inseminated, became pregnant indicating that cool hours favoured conception.

#### B. Stage of oestrus:

The analysis of data recorded on heifers and cows attending the centre for insemination revealed that out of 115 animals, reported in heat by farmers, 11.30% were in early heat while 57.39% and 31.30% were in mid and late heat, respectively (Table I). This showed that

a large percentage of cattle (42.60%) are brought for artificial insemination either too early or too late. Under the study, the percentage of animals found pregnant was the highest (54.00%) when inseminated in mid-heat whereas the percentage was lowest (21.43%) for late inseminations. However, 33.33% of the animals conceived when inseminated in the early stage of oestrus. The above findings show that better pregnancy results could be obtained by inseminating the animals in mid-heat. Similar findings have also been reported by Trimberger and Davis (1943), Son *et al.*, (1963) and Tomar (1964). Therefore, recording the time of commencement of oestrus in rural cattle for getting them served at proper time to obtain optimum fertility needs to be explained to the farmers.

#### C. Fern-pattern of cervical mucus during oestrus:

Observations on cervical mucus fern-pattern during oestrus made in 50 rural cattle in the present study revealed the percentage of typical, atypical and no pattern as 62.00%, 20.00% and 18.00% respectively (Table II). From the table it can further be observed that maximum



TABLE 2. FERN-PATTERN OF CERVICAL MUCUS IN RELATION TO PREGNANCY IN RURAL CATTLE.

No. of animals observed	Types of fern-pattern			Number pregnant		
	Typical	Atypical	No pattern	Typical	Atypical	No pattern
50	31	10	9	16	1	—
	(62.00)	(20.00)	(18.00)	(51.61)	(10.00)	(—)

Figures in parenthesis indicate percentage.

percentage i.e. 51.61% of the animals conceived which had evinced typical fern-pattern while only 10.00% of the animals evincing atypical fern-pattern conceived. However none of the animals conceived from the group showing "no-pattern". The present study, indicated that the chance of conception lies more in the animals showing typical fern-pattern of cervical mucus as compared to those showing atypical pattern. Examination of fern-pattern of cervical mucus in the cattle during oestrus should be adopted

as a routine practice. This will economise the usage of semen and enhance conception.

### Acknowledgement

The authors are grateful to Dr. S.N. Luktuke for his helpful interest, to Dr. C.M. Singh for providing facilities, to Dr. O.N. Kunzru for permission and to Dr. B.D. Gupta and K.P. Mallik for co-operation during the course of the present study.

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## Total Amino Acids of Bovine Oestrous Flow

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### ABSTRACT

The amino acid analysis was carried out on pooled samples of cervico-vaginal mucus of fertile cows in oestrus using an automatic amino acid analyser. A total of 17 amino acids were identified with glutamic acid being largest in its concentration and proline being least in its concentration.

\* \* \*

The cervical mucus has been described as a complex mixture of organic and inorganic substances and enzymes bearing a similarity to a dual gel phase system with hydration in proliferative phase and dehydration in secretory phase (Weed and Carrera, 1970). It has been recognised as a medium whose chemical structure has a profound influence on the survival of spermatozoa in the female genital tract and the fertility of female animals.

The presence of proteins in cervico-vaginal mucus has been confirmed by cytochemical techniques (Campean *et al.* 1972). The estimation of proteins in luminal fluids has been postulated as a sensitive indicator of hormonal and reproductive status of the uterus (Kulanga, 1972). However, little is known about the amino acids comprising these proteins. In the present investigation, the bovine oestrous flow has been subjected to amino acid analysis using an amino acid analyser.

### Materials and Methods

Mucus samples were aspirated from the vaginal floor of eight cows in their first or second heat following normal parturition. Equal quantity from eight samples were pooled together and mixed. One ml of the pooled cervico-vaginal mucus was hydrolysed by acid hydrolysis, viz., the material suspended in 6N HCl in a sealed test tube and heated at 110°C for 22-24 hr. The acid was removed by repeated washings with distilled water and evaporation. The volume was then made up to 2 ml with citrate buffer having a pH of 2.2. Standard amino acid mixture containing aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine and arginine was first run on the analyser. From the chromatograms the quantity of each amino acid in microgrammes per unit volume of the sample was calculated.

### Results and Discussion

The results of separation of the proteins of the cervico-vaginal mucus yielding different amino acids are presented in Table I.

In the present investigation, it was found that the cervico-vaginal mucus obtained from cows during oestrus contained three basic and 14 acidic and neutral amino acids. Thus, in all, 17



TABLE 1. TOTAL AMINO ACIDS OF BOVINE OESTROUS FLOW

Amino acid	mg/100 ml
Lysine	11.11
Histidine	4.35
Arginine	9.06
Aspartic acid	13.84
Threonine	10.48
Serine	5.88
Glutamic acid	18.82
Proline	0.44
Glycine	6.30
Alanine	8.19
Cystine	1.94
Valine	10.30
Methionine	5.96
Isoleucine	3.14
Leucine	9.59
Tyrosine	3.62
Phenylalanine	5.94
Ammonia	5.67

amino acids and ammonia were identified and estimated. Glutamic acid was found in largest concentration and was followed by aspartic acid, lysine, threonine, valine, leucine, arginine and alanine. Tryptophane was not identified in the present study due to two reasons: (1) standard used did not contain tryptophane and (2) even if tryptophane was present in the sample, it might have been des-

troyed by the process of preparation of the sample.

The available literature on the amino acids of the oestrous flow is extremely scanty and further there seems to be considerable difference of opinion among them with respect to the distribution of amino acids in the proteins of the cervical mucus. With reference to the above, Bergman and Werner (1950) and Gibbons *et al.* (1955) who have identified 13 amino acids in bovine cervical mucus employing partition paper chromatography technique and Pederson and Pommerenke (1950) who have reported as many as 17 amino acids in human cervical mucus using the same technique may be cited. The considerable difference in their results might be due to difference in the methods of approach to the problem and the technique followed; besides possible other reasons. The results of the present investigation, however, differ from those of Bergman and Werner (1950) and Gibbons *et al.* (1953) in that they have reported threonine, serine and proline were quantitatively of a higher order in bovine cervical mucus. But, it is to be noted that they identified only 13 amino acids as against 17 of them recorded in the present study.

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## Studies on some Seminal Attributes in Relation to Fertility in Crossbred Bulls

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Cross breeding programme in cattle is in progress throughout the country extensively. In the process of evolving new breeds or strains of cattle with different levels of exotic germ plasm, the use of crossbred bulls has become imminent. Exhaustive studies have been undertaken on different reproductive traits in purebred cattle. However, the work on various aspects of reproduction in crossbred bulls is in its initial stages only (Rao and Rao, 1979). The present investigation was scheduled to evaluate the crossbred bulls of 3 different genetic groups on the basis of various seminal attributes and fertility.

### Materials and Methods

Six healthy crossbred bulls maintained at the All India Co-ordinated Research Project on Cattle, Jabalpur were included in the study. The bulls belonged to 3 different genetic groups i.e.  $\frac{1}{2}$  Jersey (J)  $\times$   $\frac{1}{4}$  Holstein Friesian (HF)  $\times$   $\frac{1}{4}$  Gir (G),  $\frac{1}{2}$  HF  $\times$   $\frac{1}{4}$  J  $\times$   $\frac{1}{4}$  G and  $\frac{1}{2}$  Brown Swiss (BS)  $\times$   $\frac{1}{4}$  HF  $\times$   $\frac{1}{4}$  G, with 2 bulls in each group. The managerial practices were identical for all the bulls. Twelve semen samples were collected aseptically from each bull through artificial vagina in identical manner. Volume and pH of the semen sample was recorded immediately after collection. Spermatozoal motility, concentration, live per cent, cold shock resistance and methylene blue

reducing capacity (MBRT) were studied as detailed by Perry (1969). The conception rate of the bulls among different genetic groups was calculated on the basis of 42 pregnancies confirmed in  $\frac{1}{2}$  J  $\times$   $\frac{1}{4}$  HF  $\times$   $\frac{1}{4}$  G (16),  $\frac{1}{2}$  HF  $\times$   $\frac{1}{4}$  J  $\times$   $\frac{1}{4}$  G (17) and  $\frac{1}{2}$  BS  $\times$   $\frac{1}{4}$  HF  $\times$   $\frac{1}{4}$  G (9) cows met interse with liquid semen. Statistical analysis was done (Snedecor and Cochran, 1968) to ascertain difference between various genetic groups with regard to different seminal attributes. Correlation was studied between sperm concentration and semen volume—sperm motility—per cent live sperms—MBRT.

### Results and Discussion

The various seminal attributes and fertility of the bulls in different genetic groups are presented in Table 1. The mean semen volume was significantly more ( $P < 0.01$ ) in  $\frac{1}{2}$  BS  $\times$   $\frac{1}{4}$  HF  $\times$   $\frac{1}{4}$  G Genetic group ( $6.49 \pm 0.27$  ml) than the other groups. Spermatozoal motility (Mass and individual) was significantly better ( $P < 0.01$ ) in  $\frac{1}{2}$  J  $\times$   $\frac{1}{4}$  HF  $\times$   $\frac{1}{4}$  G and  $\frac{1}{2}$  HF  $\times$   $\frac{1}{4}$  J  $\times$   $\frac{1}{4}$  G groups as compared to  $\frac{1}{2}$  BS  $\times$   $\frac{1}{4}$  HF  $\times$  G group. The mean semen pH ranged between a narrow limit of  $6.76 \pm 0.02$  to  $6.79 \pm 0.02$  in all the groups. Sperm concentration and live sperm percentage also did not vary significantly among different genetic groups. The MBRT test significantly varied ( $P < 0.01$ ) between  $\frac{1}{2}$  HF  $\times$   $\frac{1}{4}$  J  $\times$   $\frac{1}{4}$  G ( $3.89 \pm 0.14$  mts) and



TABLE 1. VARIOUS SEMINAL ATTRIBUTES AND FERTILITY OF CROSSBRED BULLS (MEAN  $\pm$  SE)

Genetic group	Semen volume (ml)	Spermatozoala motility		Semen pH	Sperm concentration ( $\times 10^6$ /ml)	Live sperm (%)	MBRT (Mu)	Sperm cold shock resistance (%)	Conception rate (%)
		Mass (0-4)	Individual (%)						
$\frac{1}{2}J \times \frac{1}{4}HF \times \frac{1}{4}G$	5.12 $\pm$ 0.24	3.21 $\pm$ 0.06	73.33 $\pm$ 1.07	6.76 $\pm$ 0.02	1092.50 $\pm$ 55.69	87.38 $\pm$ 0.63	4.16 $\pm$ 0.17	58.22 $\pm$ 1.01	53.33 $\pm$ 0.91
$\frac{1}{2}HF \times \frac{1}{4}J \times \frac{1}{4}G$	4.09 $\pm$ 0.19	3.24 $\pm$ 0.07	73.52 $\pm$ 1.51	6.79 $\pm$ 0.02	1228.33 $\pm$ 60.87	86.42 $\pm$ 0.85	3.89 $\pm$ 0.14	61.56 $\pm$ 0.38	48.57 $\pm$ 0.39
$\frac{1}{2}BS \times \frac{1}{4}HF \times \frac{1}{4}G$	6.49 $\pm$ 0.27	2.63 $\pm$ 0.13	62.64 $\pm$ 2.59	6.79 $\pm$ 0.01	1077.22 $\pm$ 31.29	79.90 $\pm$ 1.71	7.08 $\pm$ 0.93	50.72 $\pm$ p.51	37.50 $\pm$ 0.84
All groups pooled	5.23 $\pm$ 0.23	3.02 $\pm$ 0.08	70.49 $\pm$ 1.72	6.78 $\pm$ 0.02	1132.68 $\pm$ 49.28	84.36 $\pm$ 1.06	5.04 $\pm$ 0.41	56.83 $\pm$ 0.97	46.46 $\pm$ 0.71

$\frac{1}{2}J \times \frac{1}{4}HF \times \frac{1}{4}G$  ( $4.16 \pm 0.17$  mts) groups when compared to  $\frac{1}{2}BS \times \frac{1}{4}HF \times \frac{1}{4}G$  ( $7.08 \pm 0.93$  mts). Similarly, the spermatozoa showed significantly better resistance to cold shock ( $P < 0.01$ ) in  $\frac{1}{2}HF \times \frac{1}{4}J \times \frac{1}{4}G$  ( $61.56 \pm 0.38\%$ ) and  $\frac{1}{2}J \times \frac{1}{4}HF \times \frac{1}{4}G$  ( $58.22 \pm 1.01\%$ ) groups than  $\frac{1}{2}BS \times \frac{1}{4}HF \times \frac{1}{4}G$  ( $50.72 \pm 1.51\%$ ) group. Best conception rate was evinced by  $\frac{1}{2}J \times \frac{1}{4}HF \times \frac{1}{4}G$  ( $53.33 \pm 0.91\%$ ) group followed by in  $\frac{1}{2}HF \times \frac{1}{4}J \times \frac{1}{4}G$  ( $48.57 \pm 0.39\%$ ) and  $\frac{1}{2}BS \times \frac{1}{4}HF \times \frac{1}{4}G$  ( $37.50 \pm 0.84\%$ ) groups.

Comparable results to various seminal attributes and fertility in the different genetic groups studied in the present investigation were not available. Mann (1964) described that the difference in ejaculate may vary from bull to bull as it is dependent on the functioning of accessory sex glands which are androgen dependent for their physiological activity. As found in the present study, variation in spermatozoal motility was recorded by Rao and Rao (1978) also in crossbred bulls. The semen pH was almost same in all the 3 groups, indicating apparently normal condition of the sexual apparatus of the bulls under study. More than 7.00 pH is reported to be associated with infertility in the breeding bulls, while below 6.50 pH with diseased male reproductive

tract (Juneja *et al.*, 1965). Less dehydrogenase activity present in the semen of  $\frac{1}{2}BS \times \frac{1}{4}HF \times \frac{1}{4}G$  group may be attributed to less concentration of active sperms recorded in this group.

The variation in semen volume during different seasons and interaction between various genetic groups and seasons was found to be insignificant. The effect of season on spermatozoal mass/individual motility was significant ( $P < 0.05$ ) during winter ( $2.83/65.97 \pm 1.34\%$ ) than summer ( $3.07/72 \pm 1.33\%$ ) and rainy ( $3.16/71.31 \pm 1.42\%$ ) seasons in the pooled samples of all the groups. The pH during summer ( $6.73 \pm 0.01$ ) was significantly different ( $P < 0.05$ ) than the other two seasons. The spermatozoal concentration was significantly less ( $P < 0.01$ ) during winter (888.83 million/ml) when compared to rainy (1209.72 million/ml) and summer (1300 million/ml) seasons. Interaction between breed and season was found to be nonsignificant in relation to sperm live percentage, MBRT and cold shock resistance.

Variation in spermatozoal motility was in agreement with the previous findings of Andreev (1971) in various breeds of bulls during different seasons. Significant variation in pH value between different



seasons obtained in the present study is in agreement with the findings of Kodagali (1962). The values of sperm concentration in the present investigation are significantly more in summer and rainy seasons. This may be due to increasing day length during summer as suspected by various workers. In agreement to present study, Shrivastava (1978) also did not notice significant variation between breeds and season with respect to MBRT test. Long back, Swanson and Herman (1941) described that initial spermatozoal motility and useful viability were lower in winter weather than in spring and summer conditions. He believed it to be due to the effect of adverse winter climate on the health and sexual activity of the bulls.

The correlation studies revealed positively significant relationship ( $r=0.792$ ) between spermatozoal motility and concentration. Individual motility was found to be significantly ( $P<0.05$ ) correlated with per cent live sperms positively ( $r=0.865$ ). Correlation values between sperm concentration and semen volume ( $r=0.011$ ) — MBRT ( $r=0.28$ ) were positive but nonsignificant. Shrivastava (1978) reported positive but nonsignificant correlation between sperm concentration and mass motility — MBRT.

Poor conception rate in  $\frac{1}{2}\text{BS} \times \frac{1}{4}\text{HF} \times \frac{1}{4}\text{G}$  may be attributed to poor semen quality in this genetic group. Rao and Rao (1979) reported 47.1 conception rate using  $\text{BS} \times \text{Ongole}$  and 29.5% with  $\text{HF} \times \text{Ongole}$  bulls. They observed a significant positive correlation ( $P<0.01$ ) of 0.734 between initial motility and fertility. It is concluded on the basis of various seminal attributes and conception rate obtained in the present study that the genetic groups  $\frac{1}{2}\text{J} \times \frac{1}{4}\text{HF} \times \frac{1}{4}\text{G}$  and  $\frac{1}{2}\text{HF} \times$

$\frac{1}{2}\text{J} \times \frac{1}{4}\text{G}$  were significantly better over  $\frac{1}{2}\text{BS} \times \text{HF} \times \text{G}$  group.

### Summary

Six crossbred bulls belonging to  $\frac{1}{2}\text{J} \times \frac{1}{4}\text{HF} \times \frac{1}{4}\text{G}$ ,  $\frac{1}{2}\text{HF} \times \frac{1}{4}\text{J} \times \frac{1}{4}\text{G}$  and  $\frac{1}{2}\text{BS} \times \frac{1}{4}\text{HF} \times \text{G}$  genetic groups (2 bulls per group) were evaluated on the basis of various seminal attributes and fertility. The semen pH, sperm concentration and live sperm percentage did not vary significantly among different genetic groups. Though, semen volume was significantly more in  $\frac{1}{2}\text{BS} \times \frac{1}{4}\text{HF} \times \frac{1}{4}\text{G}$  group, the spermatozoal motility, dehydrogenase activity, resistance to cold shock and fertility were significantly better in  $\frac{1}{2}\text{J} \times \frac{1}{4}\text{HF} \times \frac{1}{4}\text{G}$  and  $\frac{1}{2}\text{HF} \times \frac{1}{4}\text{J} \times \frac{1}{4}\text{G}$  genetic groups. The variation in semen volume during different seasons and interaction between various genetic groups and seasons was found to be insignificant. The effect of season on spermatozoal motility, concentration and seminal pH was significant. There was positively significant correlation between spermatozoal motility and concentration. Individual motility was found to be significantly correlated with per cent live sperms positively. Correlation values between sperm concentration and semen volume & MBRT were positive but nonsignificant.

### Acknowledgement

Thanks to Dr. H.K.B. Parekh, Sr. Scientist, A.I.C.R. Project on Cattle and Dr. B.S. Malik, Dean, College of Veterinary Science & Animal Husbandry, Jabalpur, for providing facilities to undertake the study.



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## Release of Transaminases from Buffalo Spermatozoa During Deep-Freezing\*

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### ABSTRACT

The levels of Glutamic Oxaloacetic Transaminase (GOT) before freezing (in diluted semen samples containing approx. 50 million spermatozoa) averaged 8.02 and 9.42 i.u. in Tris Fructose yolk Glycerol (TFYG) and Lactose Fructose yolk Glycerol (LFYG) diluted semen samples, which significantly ( $P < 0.01$ ) increased to 12.82 and 16.23 i.u. in freeze-thawed semen samples respectively. The difference in GOT release was significant ( $P < 0.01$ ) between dilutors, being lesser in TFGY diluent, thereby protecting spermatozoa more efficiently during freezing as compared to LFYG diluent. Glutamic Pyruvic Transaminase (GPT) levels averaged 2.67 and 3.04 in buffalo seminal plasma before freezing, which significantly ( $P < 0.01$ ) increased to 3.65 and 4.03 i.u. in TFGY and LFYG diluents after freezing respectively, the difference being non-significant between dilutors, for GPT release. It is evident that Tris dilutor is superior in protecting the spermatozoa during freezing.

\* \* \*

For evaluating semen quality and judging the efficiency of semen dilutors, the enzymatic tests have been of much value.

Effect of deep freezing on release of enzymes transaminases was studied on semen samples from five Surti buffalo bulls.

### Materials and Methods

Seventeen ejaculates from five buffalo bulls of Surti breed were used for pre-freeze and post freeze study of release of enzymes—Glutamic Oxaloacetic Transaminase (GOT) and Glutamic Pyruvate Transaminase (GPT). From the diluted samples, 1 ml. of semen (containing approx. 50 million spermatozoa) was centrifuged at 1500 r.p.m. for 15 minutes. The separated seminal plasma was maintained at 5°C in sterilized vials till the estimation of the enzymes was made. The activity of the enzymes was estimated in the seminal plasma separated from the frozen-thawed samples after one week of storage in the liquid nitrogen. The transaminases (GOT and GPT) determined by the colorimetric method according to Reitman and Frankel (1957). Analysis of variance was carried out as described by Snedecor and Cochran (1971).

### Results

The levels of GOT in seminal plasma before freezing averaged 8.02 i.u. for

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TABLE 1. AVERAGE LEVELS OF TRANSAMINASES (GOT AND GPT) BEFORE AND AFTER FREEZING IN DIFFERENT DILUTORS FOR BUFFALO SEMINAL PLASMA.

Buff. Bulls	GOT i.u./litre (in $50 \times 10^6$ spermatozoa)				GPT i.u./litre (in $50 \times 10^6$ spermatozoa)			
	Pre Freeze		Post freeze		Pre freeze		Post freeze	
I	TFYG	LFYG	TFYG	LFYG	TFYG	LFYG	TFYG	LFYG
SBAs	8.66	8.16	12.00	16.33	3.00	3.50	3.50	3.83
SBSh	6.50	8.50	12.00	14.00	3.00	3.66	4.00	4.83
SBAJ	5.83	7.33	9.33	13.33	3.00	2.83	3.50	3.50
CB <sub>1</sub>	8.25	12.00	14.50	19.00	2.00	2.50	4.00	4.50
CB <sub>2</sub>	10.87	11.12	16.25	18.50	2.37	2.75	3.25	3.5
Over all Average	8.02	9.42	12.82	16.23	2.67	3.04	3.65	4.03

Tris fructose yolk glycerol (TFYG) diluted semen samples, which increased to 12.82 and 16.23 i.u. respectively in freeze-thawed samples. (Table-1)

The levels of GPT in seminal plasma averaged 2.67 i.u. and 3.04 i.u. before freezing, which increased to 3.65 and 4.03 i.u. respectively after freezing in TFGY and LFYG diluents (Table 1) Analysis of variance revealed that the difference in GOT levels was found to be significant between temperatures as well as between dilutors, where as the difference in levels of GPT was found to be significant between temperatures but it was non-significant between the dilutors. (Table 2)

## Discussion

The loss of GOT enzyme activity from the spermatozoa and the release of the same extracellularly in seminal plasma is suggestive of sperm membrane injury (Crabo et al. 1971)

So the decrease in sperm levels of GOT concentration after freezing as observed by Buruiana et al (1975) and Jain (1979) and also the decreased levels of GPT in spermatozoa post freezing as observed by Zahariev et al (1974) are very well comparable with the results of the present experiment. The significantly more seminal plasma levels of GOT and GPT enzymes in freeze-thawed samples as

TABLE 2. ANALYSIS OF VARIANCE OF PRE-FREEZE AND POST THAW SEMINAL PLASMA LEVELS OF GOT AND GPT ENZYME IN DIFFERENT DILUTORS AND AT VARIOUS TEMPERATURES

Source of Variation	D.F.	GOT		GPT	
		M.S.	F. Value	M.S.	F. Value
Between temperature	1	168.31	31.28**	4.81	21.86**
Between dilutors	1	29.00	× 5.39**	0.72	3.27N.S.
Error	17	5.38		0.22	
Total	19				

\*\* =  $P < 0.01$

N.S. = Non Significant



compared to pre freeze levels can be compared with the observation of Roychaudhury et al (1974); who found the significant release of GOT and GPT enzymes from bull spermatozoa on cold shock.

The results of the present experiment revealed that the extracellular release of GOT in TFYG diluted semen samples was significantly lower as compared to LFYG diluted semen. (Table 1) These findings can very well be compared with those of Roychaudhury et al (1974) who did not find GOT release significantly high in Tris diluted bull semen on

cold shock. However Chinnaiya et al (1979) reported that extra cellular release of transaminases from buffalo spermatozoa on freezing was significantly lower in citric acid whey (CAW) dilutor as compared to EYCG and Tris.

### Acknowledgement

Thanks are due to the Indian Council of Agricultural Research for financial help through the award of Junior Research Fellowship to the first author during his Master's degree programme. Authors are also thankful to the Principal, Dr M.R. Patel for providing the necessary facilities for carrying out the experiment.

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# Osmotic Fragility of Erythrocytes in Periparturient Dairy Cows

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## ABSTRACT

Osmotic fragility of Red blood cells in periparturient dairy cows has been studied. The resistance of RBC to osmotic stress decreased with advancing parturition and fell maximally on the day of parturition. However, osmotic fragility of Red blood cells seemed to improve *post-partum*. Possible mechanisms which might contribute to altered susceptibility of RBC to osmotic stress near parturition have been discussed.

\* \* \*

Pregnancy and parturition are characterized by significant alterations in steroid and protein hormone profiles and certain other biochemical constituents of blood. Fluctuations in the hormone profile influence, among other things, the physiology and morphology of erythrocytes (Allen and Rasmussen, 1971). Certain steroids are known to interact with and stabilize the RBC membrane. Progesterone stabilization, for example, is believed to occur because of its interaction with membrane structural proteins (Devenuto *et al.* 1969). Addition of progesterone to stored RBC increases their osmotic resistance and minimizes haemolysis during storage (Devenuto *et al.* 1971). Diethylstilbestrol—a non-steroidal estrogen is known to cause haemolysis (Tateno and Kilbourne 1954). Oxytocin reduces the osmotic resistance of red blood cells (Singhi and Singh, 1979). Erythrocytic osmofragility

test is a measure of the resistance of erythrocytes to osmotic stress. The aim of the present investigation is to ascertain whether the erythrocytes of dairy cows show a predictable alteration in osmotic behaviour near parturition.

## Materials and Methods

Ten clinically healthy multiparous pregnant cows near parturition, from the University Dairy Farm comprised the experimental animals. Five ml of venous blood was collected into heparinized vials from each cow on three consecutive days before parturition ( $-D_3$ ,  $-D_2$ ,  $-D_1$ ), on the day of parturition ( $D_0$ ) and on three consecutive days *post-partum* ( $D_1$ ,  $D_2$ ,  $D_3$ ). The blood samples collected at ambient temperature were carried to the laboratory as soon as possible, sedimented for 10 minutes at room temperature and centrifuged for 10 minutes at 2000 rpm. After the separation of plasma the packed red cells were stored overnight in a refrigerator at 4°C (Jain, 1973). The osmotic fragility was determined as per the method described by Jain (*loc. cit.*). Exactly 0.02 ml of unwashed blood cells was added to 5.0 ml of buffered NaCl solution in concentrations ranging from 0.40% to 0.90% and to 5.0 ml of double distilled water. The contents were gently mixed and allowed to stand for 30 minutes at room temperature before centrifuging at 2000 rpm for 5 minutes. The haemoglobin content of



TABLE RESULTS OF ERYTHROCYTIC OSMOTIC FRAGILITY TEST IN PERIPARTURIENT DAIRY COWS.

	$-D_3$	$-D_2$	$-D_1$	$D_0$	$D_1$	$D_2$	$D_3$
Beginning haemolysis (% NaCl)	0.80-0.85	0.80-0.85	0.85	0.90	0.90	0.75-0.78	0.75-0.78
Complete haemolysis (% NaCl)	0.40	0.40	0.45-0.50	0.50-0.55	0.50-0.55	0.40-0.45	0.40-0.45
Mean corpuscular fragility (% NaCl)	0.53	0.54	0.54	0.60	0.58	0.55	0.55

the supernatant was determined spectrophotometrically at 540  $\mu$ m. The percentage of haemolysis in each tube was calculated assuming cent per cent haemolysis in distilled water. The 'beginning haemolysis', 'complete haemolysis' and the 'mean corpuscular fragility' (i.e. 50% haemolysis) were determined as per Suess *et al.* (1948).

### Results

Presented in the Table is summary of the results. The 'beginning haemolysis' on  $-D_3$ ,  $-D_2$  and  $-D_1$  of parturition occurred at relatively hypotonic saline concentration. However, erythrocytes showed increased osmotic fragility on  $D_0$  (day of parturition) and  $D_1$  of parturition with 'beginning haemolysis' occurring in physiological saline concentration. 'Complete haemolysis' on  $-D_3$  and  $-D_2$  occurred at 0.40% NaCl. However, on the day of calving ( $D_0$ ) the erythrocytes became less resistant to osmotic stress and 'complete haemolysis' occurred at relatively higher saline concentrations (0.50 – 0.55%). This trend persisted on the subsequent day ( $D_1$ ).

The resistance of RBC to osmotic stress increased on  $D_2$  and  $D_3$  post-partum with 'beginning haemolysis' occurring between 0.75 and 0.78% NaCl and 'complete haemolysis' between 0.40% and 0.45% NaCl. The 'mean corpuscular fragility' (MCF) on  $D_0$  was observed at 0.60% NaCl as compared to that on  $-D_3$ ,

$-D_2$  and  $-D_1$  when the mean corpuscular fragility occurred at 0.53%, 0.54% and 0.54% NaCl respectively. The MCF on  $D_1$ ,  $D_2$  and  $D_3$  post-partum occurred at the saline strength of 0.58%, 0.55% and 0.55% respectively.

The shape of the fragility curve tended to be monophasic on  $-D_1$  and  $D_0$  as against its biphasic or multiphasic shape on  $-D_3$  or  $D_3$  around parturition.

### Discussion

The data in the present study provide evidence that the erythrocytes are under considerable stress near parturition in cows. Osmotic resistance of red blood cells in the present study decreased slowly towards parturition and fell maximally on the day of parturition. Since the periparturient period is characterised by enormous fluctuations in steroid and protein hormone levels, the changes in the RBC membrane physiology are likely to occur and these changes might be reflected through altered susceptibility of RBC membrane to osmotic stress. The slow withdrawal of the progesterone coupled with enormous release of estrogens and oxytocin might decrease the resistance of RBC to osmotic stress. Oxytocin by virtue of its antidiuretic and saluretic action may increase osmotic fragility of erythrocytes (Karim and Assali, 1961). The precise nature of the chemical lesion inflicted on RBC membrane leading



to reduced osmotic resistance of RBC is unknown. In certain forms of haemolytic anaemias (e.g. "Xerocytosis") there is a decrease in RBC 2, 3, diphosphoglyceride (2, 3 DPG) (Fairbanks *et al.* 1978). The levels of 2, 3, DPG in the erythrocytes are increased upon androgen treatment (Parker *et al.* 1972) and also during later stages of pregnancy (Rorth and Bille Brahe, 1972). Both androgens and progesterone are known to stabilize RBC membrane. It may, therefore, be construed that the withdrawal of progesterone and release of estrogens and oxytocin near parturition may interfere with 2, 3, DPG metabolism of RBC and make them osmofragile. Alternatively, the changes in the calcium metabolism during pregnancy might also affect RBC calcium. It is not known whether estrogens and/or oxytocin bring about any structural and physiological changes by altering  $Ca^{2+}$  metabolism in red cell membrane.

The monophasic shape of the fragility curve before parturition may be indicative of hemosuppressive effect of estrogen (Siegal, 1970), which prevents the flow of immature red cells (reticulocytes) from the bone marrow into blood stream, thus leaving into circulation only the mature RBC of uniform thickness and age. Reticulocytes tend to be more osmoresistant (Suess *et al. loc. cit.*). The withdrawal of inhibitory effect on haemopoiesis may account for multiphasic fragility curve *post-partum*.

### Acknowledgements

The authors are thankful to Dr M.S. Tiwana, Senior Geneticist and Incharge, P.A.U. Dairy Farm, and Professor-cum-Head, Department of Animal Science, Punjab Agricultural University, Ludhiana for their kind cooperation.

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## Freezeability, GOT Release and Fertility of Crossbred Bulls Semen

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Differences in semen characteristics due to various levels of exotic blood under Indian conditions needs thorough investigation before starting freezing of their semen on large scale. Exhaustive studies have been undertaken on reproductive capability of purebred bulls, though in crossbred bulls it is in initial stages only. Razdan and Gupta (1980) recommended that the seminological studies including semen freezing are needed among the various breeds of bulls with 75% exotic blood. Biochemical nature of semen between different genetic group of bulls can give valuable information on semen quality and its fertilizing capacity. The leakage of cellular components into extracellular medium provides a possible test of cell damage for spermatozoa, the glutamic oxalacetic transaminase (GOT) release being the best indicator of cell damage (Brown *et al.*, 1971). The present investigation was aimed to evaluate the crossbred bulls of 3 different genetic groups (75% exotic blood) on the basis of spermatozoal motility, semen freezeability and fertility. The extent of withstanding the cell damage caused by semen dilution, glycerolization and freezing—thawing of the bulls of different genetic groups was measured by estimating the GOT release by the sperms.

### Materials and Methods

The study was conducted on 6 healthy

bulls maintained at the All India Co-ordinated Research Project on Cattle, Jabalpur. The bulls belonged to 3 different genetic groups i.e.  $\frac{1}{2}$  Jersey (J)  $\times$   $\frac{1}{4}$  Holstein Friesian (HF)  $\times$   $\frac{1}{4}$  Gir (G),  $\frac{1}{2}$  HF  $\times$   $\frac{1}{4}$  J  $\times$   $\frac{1}{4}$  G and  $\frac{1}{2}$  Brown Swiss (BS)  $\times$   $\frac{1}{4}$  HF  $\times$   $\frac{1}{4}$  G, with 2 bulls in each group. The managerial practices were identical throughout the study. Twelve semen samples were collected aseptically from each bull through artificial vagina. Routine semen examination was done for spermatozoal motility, concentration, live and abnormal percentage. The semen samples with optimum qualities were diluted (1:10) in conventional egg yolk citrate diluter, which was then glycerolized (7% glycerol), equilibrated (5 hours) and frozen in liquid nitrogen as described earlier (Pandit *et al.*, 1980).

The individual motility of the spermatozoa was estimated before glycerolization, and after glycerolization, equilibration and thawing of frozen semen. The GOT was estimated in fresh, glycerolized and post thaw samples in seminal plasma after Yatizidis (1960). The conception rate of the bulls of different genetic groups were worked out on the basis of 42 pregnancies confirmed in  $\frac{1}{2}$  J  $\times$   $\frac{1}{4}$  HF  $\times$   $\frac{1}{4}$  G (16),  $\frac{1}{2}$  HF  $\times$   $\frac{1}{4}$  J  $\times$   $\frac{1}{4}$  G (17) and  $\frac{1}{2}$  BS  $\times$   $\frac{1}{4}$  HF  $\times$   $\frac{1}{4}$  G (9) cows met interse with liquid semen. Statistical analysis was employed (Snedecor and Cochran, 1968) to know the difference in the various genetic

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TABLE 1. SPERMATOZOAL MOTILITY (%) AND GOT RELEASE (UNITS/10<sup>9</sup> SPERMATOZOA) AFTER COLLECTION, GLYCEROLIZATION AND FREEZING-THAWING OF SEMEN WITH FERTILITY PERCENTAGE (MEAN±S.E.).

Genetic group	After collection		After glycerolization		After freezing-thawing		Conception rate.
	Motility	GOT	Motility	GOT	Motility	GOT	
$\frac{1}{2}J \times \frac{1}{4}HF \times \frac{1}{4}G$	73.33±1.07	371.91±12.39	62.08±2.98	498.64±24.82	38.75±2.31	976.61±54.03	53.33
$\frac{1}{2}HF \times \frac{1}{4}J \times \frac{1}{4}G$	73.53±1.51	406.32±15.01	61.25±1.25	619.12±35.07	38.75±1.95	979.07±53.42	48.57
$\frac{1}{2}BS \times \frac{1}{4}HF \times \frac{1}{4}G$	62.64±2.59	377.49±8.39	56.25±3.26	607.97±41.02	28.33±3.55	1043.84±51.35	37.50
All groups pooled	69.83±1.08	385.25±7.29	59.86±1.73	575.24±21.31	35.28±2.13	999.84±30.15	47.19

groups in relation to fertility, sperm motility and GOT release, and effect of glycerolization and semen freezing—thawing on sperm motility and GOT release. The correlation was studied between GOT release and sperm motility—sperm concentration—abnormal sperms.

### Results and Discussion

The mean spermatozoal motility (%) and GOT release (Units/10<sup>9</sup> spermatozoa in supernatant) after collection, glycerolization and freezing—thawing of the semen with fertility percentage of the bulls of different genetic groups are presented in Table 1.

The mean individual sperm motility pooled for all the bulls in fresh semen (69.83±1.08) deteriorated significantly ( $P<0.01$ ) after glycerolization (59.86±1.73) and freezing (35.28±2.13). However, the GOT in the supernatant showed an increasing trend in the fresh (385.25±7.29), glycerolated (575.24±21.31) and frozen (999.84±30.15) samples. There was not much difference in the fresh semen spermatozoal motility of  $\frac{1}{2}J \times \frac{1}{4}HF \times \frac{1}{4}G$  (73.33±1.07) and  $\frac{1}{2}HF \times \frac{1}{4}J \times \frac{1}{4}G$  (73.53±1.51) genetic group of bulls, but it was significantly low ( $P<0.05$ ) in

$\frac{1}{2}BS \times \frac{1}{4}HF \times \frac{1}{4}G$  (62.64±2.59) genetic group. Similarly, the motility in the later group was less than other groups after glycerolization (56.25±3.26) and freezing (28.33±3.55) with maximum GOT release following freezing (1043.84±51.35). This indicated breed difference in the freezeability of the spermatozoa. The sperms of the  $\frac{1}{2}BS \times \frac{1}{4}HF \times \frac{1}{4}G$  group of bulls were found to be more susceptible to damage by freezing as indicated by poor post-thaw spermatozoal motility with maximum GOT release when compared to other 2 groups. The correlation established between GOT release and sperm mass motility (0.19)—individual motility (0.362) was positively non-significant. Similarly, the correlation between GOT release and sperm concentration (−0.265)—abnormal sperms (−0.006) was negatively nonsignificant.

Comparable results on the genetic groups studied in the present investigation were not available. However, several workers have indicated that the freezeability of the spermatozoa obtained from the different breed of bulls varies. Presence of GOT in the bull seminal plasma was observed earlier also (Flipse, 1960). the GOT activity was reported to increase



when semen was diluted with extenders, equilibrated and frozen. Graham and Pace (1967) reported that plunging spermatozoa in liquid nitrogen destroyed the cell motility and apparently damaged cell membrane to cause an increase in the concentration of seminal plasma GOT.

The percent conception rate in the present study by interse mating was best in  $\frac{1}{2}J \times \frac{1}{4}HF \times \frac{1}{4}G$  group (53.33) followed by in  $\frac{1}{2}HF \times \frac{1}{4}J \times \frac{1}{4}G$  group (48.57). It was poorest in  $\frac{1}{2}BS \times \frac{1}{4}HF \times \frac{1}{4}G$  (37.50) group. Rao and Rao (1979) reported 47.1% conception rate using BS  $\times$  Ongole and 29.5% with HF  $\times$  Ongole bulls. They observed a significant positive correlation ( $P < 0.01$ ) of 7.734 between initial motility and fertility. It is concluded from the present investigation that  $\frac{1}{2}J \times \frac{1}{4}HF \times \frac{1}{4}G$  and  $\frac{1}{2}HF \times \frac{1}{4}J \times \frac{1}{4}G$  genetic groups were superior over  $\frac{1}{2}BS \times \frac{1}{4}HF \times \frac{1}{4}G$  group, as indicated by poor sperm motility, low freezeability and more cellular damage in the later group.

### Summary

The study was conducted on 6 cross-bred bulls belonging to 3 different genetic groups with equal number of bull in each group. The mean individual sperm motility (%) in fresh semen ( $69.83 \pm 1.08$ )

deteriorated significantly ( $P < 0.01$ ) after glycerolization ( $59.85 \pm 1.73$ ) and freezing ( $35.28 \pm 2.13$ ). However, the GOT in the supernatant (Units  $10^9$  spermatozoa) showed an increasing trend in the fresh ( $385.25 \pm 7.29$ ), glycerolated ( $575.24 \pm 21.31$ ) and frozen ( $999.84 \pm 30.15$ ) samples. The sperm motility in  $\frac{1}{2}BS \times \frac{1}{4}HF \times \frac{1}{4}G$  genetic group was less with maximum GOT release following glycerolization and freezing when compared to  $\frac{1}{2}J \times \frac{1}{4}HF \times \frac{1}{4}G$  and  $\frac{1}{2}HF \times \frac{1}{4}J \times \frac{1}{4}G$  groups. The correlation between GOT and sperm mass motility (0.19)—individual motility (0.362) was positively nonsignificant, while it was negatively nonsignificant between GOT release and sperm concentration ( $-0.265$ )—abnormal sperms ( $-0.006$ ). The percent conception rate was best in  $\frac{1}{2}J \times \frac{1}{4}HF \times \frac{1}{4}G$  (53.53) followed by in  $\frac{1}{2}HF \times J \times G$  (48.57) group and was poorest in  $\frac{1}{2}BS \times \frac{1}{4}HF \times \frac{1}{4}G$  (37.50) genetic group.

### Acknowledgement

Thanks to Dr. H.K.B. Parekh, Senior Scientist of the project and Dr. B.S. Malik, Dean, College of Veterinary Science & A.H., Jabalpur, for providing facilities to undertake the study.

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## Parturition in Goats

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### ABSTRACT

The process of parturition was observed in 27 Malabari, 35 Alpine  $\times$  Malabari and 23 Saanen  $\times$  Malabari does. The prominent signs of approaching parturition were tumefaction of Vulva, udder engorgement and relaxation of pelvic ligaments. These symptoms were observed for the first time at 7th day prior to kidding. Initially, the signs were moderate but there was gradual and conspicuous increase in the intensity of these signs on approaching parturition. Colostrum was present in the udder two days before parturition. Visible flow of cervical mucus was evident 24 to 48 hours before kidding. The duration of 1st, 2nd and 3rd stage of parturition and the total duration was  $201.46 \pm 28.02$ ,  $21.34 \pm 2.03$ ,  $145.79 \pm 15.25$  and  $364.84 \pm 27.20$  minutes respectively in single birth and  $199.09 \pm 22.39$ ,  $30.94 \pm 2.22$ ,  $131.84 \pm 9.98$  and  $361.68 \pm 27.45$  minutes respectively in multiple births. The total duration and the duration of different stages of parturition was not at variance between genetic groups. The weight of the kid was positively correlated with placental weight both in single and multiple births.

\* \* \*

### Materials and Methods

Eightyfive does, comprising 27 Malabari, 35 Alpine  $\times$  Malabari and 23 Saanen  $\times$  Malabari, of the All India Co-ordinated Research Project on Goats, Mannuthy, formed the materials for the

study. One month before the expected day of kidding, the does were watched thrice for prepartum changes. The duration of parturition was arbitrarily divided into three stages. The period from the beginning of labour pains to the rupture of the first water bag was taken as the first Stage of labour. The period from the rupture of first water bag to the expulsion of the foetus was taken as the Second stage in single birth and in multiple births that from the rupture of the first water bag to the expulsion of the last foetus. The duration of the second stage in single birth was, in sequence, from the rupture of the first water bag to the appearance of the amniotic sac (phase 1) from the appearance of the amniotic sac to the appearance of the hooves and muzzle in the intact amnion (phase 2), from the appearance of the hooves and muzzle to the rupture of the amniotic sac (phase 3), and from the rupture of the amniotic sac to the expulsion of the foetus (phase 4). The interval between the expulsion of the foetus and the expulsion of the foetal membranes in single birth and that between the expulsion of the last foetus and the complete expulsion of the foetal membranes in multiple births formed the 3rd stage. The data were subjected to statistical analysis (Senedecor and Cochran, 1967).

### Results and Discussion

Tumefaction of the Vulva, engorgement of the udder and relaxation of the



pelvic ligaments were the prominent prepartum changes. The visible symptoms were noticed for the first time about a week before kidding. The intensity of these changes, slight to moderate in the beginning, increased gradually with the advancement of the kidding date. On the day before kidding, most of the does exhibited marked vulval tumefaction, udder engorgement and relaxation of pelvic ligaments. Colostrum was present four days before kidding in 30 (35.29%) does, and in the rest two days before kidding. Visible flow of cervical mucus was detected 48 hours before kidding in 75 does, and 24 hours before kidding in the rest. These observations are in general agreement with those reported in different breeds of does by earlier workers (Roberts, 1971; Arthur, 1975).

The first stage of parturition found to last from 55 to 335 minutes with a mean of  $201.46 \pm 28.02$  minutes in single birth and from 65 to 345 minutes with a mean of  $199.09 \pm 22.39$  minutes in multiple births. The second stage averaged  $21.34 \pm 2.03$  minutes in single birth and  $30.04 \pm 2.22$  minutes in multiple births. The duration of the third stage ranged from

50-290 minutes with a mean of  $145.79 \pm 15.25$  minutes in single birth and from 80 to 280 minutes with a mean of  $131.84 \pm 9.98$  minutes in multiple births. The total duration of parturition was  $364.84 \pm 27.20$  minutes in single birth and  $361.68 \pm 27.45$  minutes in multiple births. The durations of the stages and the total duration were not at variance between the three genetic groups. The interval between the births of two consecutive foetuses was  $12.12 \pm 0.88$ ,  $12.90 \pm 0.95$  and  $11.82 \pm 0.90$  minutes in Malabari, Alpine  $\times$  Malabari and Saanen  $\times$  Malabari goats, respectively. The mean weight of the foetal membranes and weight of the kid was  $233.26 \pm 12.63$  (180–365) g and  $2.16 \pm 0.18$  (0.6–3.50) kg in single birth and  $357.57 \pm 13.52$  (280–440) g and  $3.51 \pm 0.36$  (1.4–7.0) kg in multiple births respectively. The weights of the foetal membranes and the kid were both positively correlated in single and multiple births in all the genetic groups.

### Acknowledgement

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

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## Biometrics of Pelvis in Bannur Ewes and Surti Does<sup>1</sup>

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### ABSTRACT

The pelvic norms were worked out in 5 Bannur ewes and 9 Surti does belonging to Unit No. 22 of Bombay Veterinary College. All these animals were adult and normally eutocic.

The external pelvic measurements were recorded, so also various diameters of outlet including weight of pelvis. For inlet, conjugate, superior bis-iliac, inferior bis-iliac, vertical and oblique diameters and for outlet, vertical and transverse diameters were recorded. The area of inlet, shape of inlet and pelvic index were also worked out.

Regression equation  $Y = a - bX$  has been worked out indicating the correlation between the external and internal measurements in Bannur ewes and Surti does.

The formulae derived for cattle to carry out internal pelvimetry from external measurements can not be applied in Bannur ewes and Surti does but required a slight modification since the actual diameters of pelvis were significantly greater than the estimated diameters. The studies clearly indicated the variations in the diameters due to species. Modified formulae have been suggested to estimate diameters

from the external measurements in Bannur ewes and Surti does.

\* \* \*

Selection of proper type of sheep and goats for breeding purpose based on size of pelvic opening reduces the risk of dystocia to a certain extent. Crossbreeding between large mutton type breeds with small sized breeds of sheep and goats leads to increase in birth weights of lambs and kids with consequent dystocia. It has been well established that, there is a positive correlation between lambs birth weight and the occurrence of dystocia (Gunn, 1968; Hight and Jury, 1970).

Differences in pelvic diameters between eutocic and dystocic sheep have been reported by Naaktgeboren *et al.* (1971). Quinlivan (1971) and Fogarty (1974).

The study of pelvimetry has achieved great importance in medical field (William; 1939; Herbert, 1940 and Baird, 1952). Lot of efforts are being made to study the same in equines (Mia, 1973) in bovines, (Hadi, 1963) and in crossbred Cattle (Reddy, 1972). The work of similar nature in ovines and caprines is too scarce. The present paper deals with the bio-

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metrics of pelvis in Bannur ewes and Surti does.

### Materials and Method

Five Bannur ewes and 9 Surti does belonging to Livestock Farm, Aarey Milk Colony (Unit No. 22) of the Bombay Veterinary College were included for the study. All these animals were healthy, adult and normally eutocic.

The animals were weighed and the external measurements were recorded with the help of "Pelvimeter" devised by Hadi and Sane (1963) with the little modifications (Plate I) and vernier callipers while internal measurements were recorded by using measuring scale and non elastic thread.

The distance between the two external angles of ilia (A), the distance between the two lateral ischial tuberosities (B) and the perpendicular distance between the coxo-femoral joint and the highest point of croup (C) were recorded.

The animals were slaughtered and the entire bony pelvis along with sacrum and first two coccygeal vertebrae were dissected out. Soft tissues were separated from the points to be measured and actual measurements were recorded. Pelvis were boiled in 20% NaOH solution for 20 to 25 minutes to digest the remaining tissues. Pelvis along with sacrum and first two coccygeal vertebrae were weighed and preserved for further study.

For pelvic outlet (Plate II), the distance between summit of ischial arch and the point of articulation between first and second coccygeal vertebra (Vertical/ Superior-inferior diameter), the distance between the lateral tuberosities of tuber ischia (Transverse diameter), were recorded with the help of measuring scale and thread.

Similarly for pelvic inlet (Plate III) the distance from the centre of promontory to the anterior margin of pubic symphysis ('C'—Conjugate/Sacropubic diameter) was recorded. The transverse diameter (Superior transverse 'T<sub>1</sub>') was measured at upper third of pelvic inlet whereas transverse diameter (Inferior transverse 'T<sub>2</sub>') was measured at it's greatest width just above psoas tubercles. The vertical diameter was measured between anterior part of pubic symphysis and the point of articulation of the third and fourth sacral vertebra. Distance from sacro iliac joint of one side through the centre of pelvic cavity to the psoas tubercle of opposite side constituted the oblique diameter. The value of the product of conjugate and the transverse diameter (C×T<sub>2</sub>) was recorded as an area of pelvic inlet whereas the value of the ratio of conjugate diameter to the transverse diameter ( $\frac{C}{T_2}$ ) constituted shape of inlet. Pelvic index was derived by using the formula  $\frac{C \times 100}{T_2}$  (Sharma, 1964).

To estimate the dimensions of outlet and inlet of pelvis, the formulae adopted by Arloing (1968) and Chauveau (1891) for cattle were used.

All the data were subjected to statistical analysis as per Snedecor and Cochran (1967).

### Results and Discussion

#### I. Pelvic Norms:

The norms of pelvic diameters as observed in Bannur ewes and Surti does are presented in Table 1.

The table reveals that the average transverse and vertical diameters of outlet in Bannur ewes were  $4.98 \pm 0.42$  and  $6.48 \pm 0.54$  cm respectively. The average



TABLE 1.

## NORMS OF PELVIMETRY IN BANNUR EWES AND SURTI DOES

(All measurements are given in cm.)

Pelvic Measurements	Bannur ewes (n = 5)					Surti does (n = 9)				
	Mean	S.D.	S.E.	Range	C.V. %	Mean	S.D.	S.E.	Range	C.V. %
<i>Diameters of Outlet</i>										
Transverse	4.98	0.93	0.42	4.0-6.2	18.64	4.53	0.41	0.14	3.9-5.1	9.07
Vertical	6.48	1.20	0.54	4.8-8.0	18.49	7.12	0.65	0.22	6.6-8.5	9.15
<i>Diameters of Inlet</i>										
Superior bis-iliac $T_1$	6.18	0.61	0.27	5.5-7.1	9.93	5.52	0.32	0.11	5.1-5.9	5.70
Inferior bis-iliac $T_2$	6.96	1.01	0.15	5.6-7.7	14.44	6.65	0.62	0.21	6.0-7.5	9.37
Cojugate C	10.18	1.19	0.53	8.5-11.5	15.14	11.03	1.49	0.50	9.5-13.3	13.54
Vertical	6.96	1.27	0.57	5.0-8.2	18.18	7.13	0.45	0.15	6.7-8.0	6.00
Oblique	8.18	0.59	0.26	7.2-8.7	7.20	8.42	0.94	0.31	7.2-9.7	11.18
Area of Inlet $C \times T_2$ cm <sup>2</sup>	65.96	17.26	7.72	47.16-81.4	26.17	14.09	15.73	5.24	57.00-99.00	21.23
Shape of Inlet $\frac{C}{T_2}$	1.46	0.16	0.07	1.23-1.67	10.85	1.65	0.11	0.04	1.48-1.84	6.36
Pelvic Index $\frac{C \times 100}{T_2}$	146.0	16.00	7.00	123.0-167.0	10.85	165.00	11.00	4.00	148.00-184.00	6.36
Weight of Pelvis in g.	92.00	30.54	13.63	55.0-125.0	33.19	80.56	17.75	5.92	70.00-120.00	22.03



TABLE 2. EXTERNAL BODY MEASUREMENTS AND ACTUAL DIAMETERS OF PELVIS (cm).

Items	Symbol	Bannur ewes (Mean)	Surti does (Mean)
1. Distance between the two external angles of ilia	$X_1$	10.64	9.41
2. Distance between the two lateral ischial tuberosities	$X_2$	4.28	3.55
3. Distance between the hip-joint to croup	$X_3$	7.66	7.69
4. Vertical diameter of inlet	$Y_1$	6.96	7.13
5. Transverse diameter of inlet	$Y_2$	6.98	6.65
6. Conjugate diameter of inlet	$Y_3$	10.18	11.03
7. Vertical diameter of outlet	$Y_4$	6.48	7.12
8. Transverse diameter of outlet	$Y_5$	4.98	4.53

superior bis-iliac, inferior bis-iliac, conjugate, vertical and oblique diameters of inlet were  $6.18 \pm 0.27$ ,  $6.96 \pm 0.45$ ,  $10.18 \pm 0.53$ ,  $6.96 \pm 0.57$  and  $8.18 \pm 0.26$  cm respectively. The area of inlet was  $65.96 \pm 7.72$  cm<sup>2</sup>. The shape of inlet and pelvic index were  $1.46 \pm 0.07$  and  $146.0 \pm 7.0$  cm respectively. The pelvis weighed  $92.0 \pm 13.63$  g.

Similarly in Surti does, the mean values of transverse and vertical diameters of outlet were  $4.53 \pm 0.14$  and  $7.12 \pm 0.22$  cm respectively. The superior bis-iliac, inferior bis-iliac, conjugate vertical and oblique diameters of inlet were  $5.52 \pm 0.11$ ,  $6.65 \pm 0.21$ ,  $11.03 \pm 0.50$ ,  $7.13 \pm 0.15$  and  $8.42 \pm 0.31$  cm respectively. The area of inlet was  $74.09 \pm 5.24$  cm<sup>2</sup>. The shape of inlet and pelvic index was  $1.65 \pm 0.04$  and  $165.0 \pm 4.0$  respectively. The weight of pelvis was found to be  $80.56 \pm 5.92$  g.

The biological data of the type of the present study consists of a sample of non homogenous nature. It is subjected to variations due to various factors comprising effect of nutrition, age, climate, heredity, gestation and breeding performance. The present study aimed at esti-

imating various external and internal diameters of pelvis in Bannur ewes and Surti does. The measurements as observed in present study are lesser than those reported by Rainard (1845), Roberts (1971), Fogarty (1974), Sisson and Grossman (1975) and McSporran (1979) in sheep and Rainard (1845) in goats. This is most probably on account of breed differences. The Bannur ewes and Surti does are of smaller size having lower body weights as compared to that of exotic breeds like Merino.

## II. Correlation between external body measurements and actual diameters of pelvis:

For this purpose items presented in Table 2 were selected.

### Correlation coefficients:

Table 3 shows the correlation coefficients between all the possible combinations. Out of 22 combinations in Bannur ewes, 6 combinations ( $X_2Y_1$ ,  $X_2Y_3$ ,  $X_2Y_4$ ,  $X_3Y_3$ ,  $X_4Y_3$  and  $X_5Y_3$ ) were significantly correlated while one combination ( $Y_1Y_4$ ) vertical diameter of inlet and vertical diameter of outlet was highly



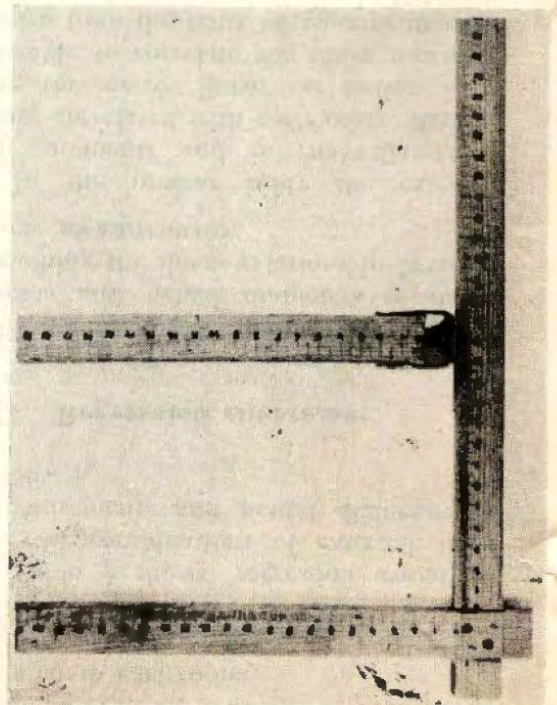
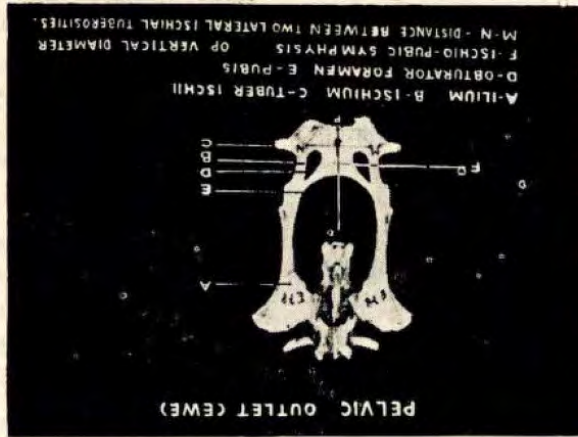




TABLE 3. VALUES OF CORRELATION COEFFICIENTS (cm)

Sr. No.	Items		Bannur ewes (n = 5)	Surti does (n = 9)
1.	X <sub>1</sub>	Y <sub>1</sub>	-0.5385 NS	0.6521 NS
2.	X <sub>1</sub>	Y <sub>2</sub>	-0.0024 NS	0.7664 *
3.	X <sub>1</sub>	Y <sub>3</sub>	-0.6864 NS	0.7600 *
4.	X <sub>1</sub>	Y <sub>4</sub>	-0.5422 NS	0.7897 *
5.	X <sub>1</sub>	Y <sub>5</sub>	-0.1391 NS	0.2783 NS
6.	X <sub>2</sub>	Y <sub>1</sub>	0.8914 *	0.4411 NS
7.	X <sub>2</sub>	Y <sub>2</sub>	0.8345 NS	0.9258 **
8.	X <sub>2</sub>	Y <sub>3</sub>	0.9049 *	0.2117 NS
9.	X <sub>2</sub>	Y <sub>4</sub>	0.8868 *	0.1096 NS
10.	X <sub>2</sub>	Y <sub>5</sub>	0.4390 NS	0.0001 NS
11.	X <sub>3</sub>	Y <sub>1</sub>	0.7531 NS	0.7555 *
12.	X <sub>3</sub>	Y <sub>2</sub>	0.6343 NS	0.8227 **
13.	X <sub>3</sub>	Y <sub>3</sub>	0.9189 *	0.9610 **
14.	X <sub>3</sub>	Y <sub>4</sub>	0.7824 NS	0.6232 NS
15.	X <sub>3</sub>	Y <sub>5</sub>	0.1431 NS	0.3761 NS
16.	Y <sub>1</sub>	Y <sub>4</sub>	0.9761 **	0.5332 NS
17.	Y <sub>2</sub>	Y <sub>5</sub>	0.7275 NS	0.4194 NS
18.	Y <sub>4</sub>	Y <sub>5</sub>	0.9192 *	0.6489 NS
19.	X <sub>1</sub> +X <sub>2</sub>	X <sub>3</sub>	-0.0934 NS	0.7487 *
20.	X <sub>1</sub>	X <sub>4</sub>	0.7281 NS	0.1740 NS
21.	X <sub>1</sub>	X <sub>5</sub>	-0.4279 NS	0.8284 **
22.	X <sub>2</sub>	X <sub>5</sub>	0.9487 *	0.0020 NS

Table value:

n = Number of bivariate observations (X,Y)			
n	D.F.	0.05	0.01
	n-2		
5	3	0.8783	0.9587
9	7	0.6664	0.7977

correlated. The anterior width or distance between the two external angles of ilia (X<sub>1</sub>) was negatively correlated to all combinations. Rest of the combinations were non significant.

Out of 22 combinations in Surti does, 5 combinations (X<sub>1</sub>Y<sub>2</sub>, X<sub>1</sub>Y<sub>3</sub>, X<sub>1</sub>Y<sub>4</sub>, X<sub>2</sub>Y<sub>1</sub> and X<sub>1</sub> + X<sub>2</sub>X<sub>3</sub>) were significantly correlated while 4 combinations (X<sub>2</sub>Y<sub>2</sub>, X<sub>3</sub>Y<sub>2</sub>, X<sub>3</sub>Y<sub>3</sub> and X<sub>1</sub>Y<sub>3</sub>) X<sub>1</sub>Y<sub>3</sub> were highly correlated. Rest of the combinations were non significant.

The present data indicated that pelvis are tending to increase in every direction

and that various diameters are complexly related to each other.

### Regression Equations:

Table 4 shows regression equations for all combinations of external body measurements and actual diameters of pelvis.

### III. Regression equations:

Table 4 shows regression equations for all combinations of external body measurements and actual diameters of pelvis assuming the linear relationship between these measurements.

In the present study the external measurements and internal diameters were correlated with each other. Assuming the simple linear correlation it is possible to correlate the inner measurements from the outer measurements with the help of regression equations. Although such regression equations were presented by Hadi (1963) in Murrah buffaloes, similar work was not available for comparison in sheep and goats.

### IV. Testing of formulae used for estimation of pelvic diameters outlet and inlet:

The external measurements viz anterior width (A), posterior width (B) and height (C) were used to estimate the pelvic diameters. Actual diameters of pelvis were recorded after the animals were slaughtered. Comparison between estimated and actual diameters was made by using Students 't' test and is presented in table 5.

Table 5 reveals that the transverse diameter of outlet in Bannur ewes was significant while the vertical diameter was non significant. The transverse and



TABLE 4. VALUES OF REGRESSION EQUATIONS (cm)

Sr. No.	Items		Bannur ewes (n=5)		Surti does (n=9)	
			b	X + a	b	X + -a
1.	X <sub>1</sub>	Y <sub>1</sub>	-0.4657	X <sub>1</sub> + 11.9150	0.2387	X <sub>1</sub> + 4.8838
2.	X <sub>1</sub>	Y <sub>2</sub>	-0.0014	X <sub>1</sub> + 6.9949	0.3922	X <sub>1</sub> + 2.9585
3.	X <sub>1</sub>	Y <sub>3</sub>	-0.5602	X <sub>1</sub> + 16.1405	0.9328	X <sub>1</sub> + 2.2524
4.	X <sub>1</sub>	Y <sub>4</sub>	-0.4440	X <sub>1</sub> + 11.2024	0.4181	X <sub>1</sub> + 3.1857
5.	X <sub>1</sub>	Y <sub>5</sub>	-0.0882	X <sub>1</sub> + 5.9184	0.0939	X <sub>1</sub> + 3.6464
6.	X <sub>2</sub>	Y <sub>1</sub>	-2.1444	X <sub>2</sub> - 2.1980	0.5458	X <sub>2</sub> + 5.1924
7.	X <sub>2</sub>	Y <sub>2</sub>	1.5939	X <sub>2</sub> + 0.1581	1.6016	X <sub>2</sub> + 0.9643
8.	X <sub>2</sub>	Y <sub>3</sub>	2.0542	X <sub>2</sub> + 1.3880	0.8781	X <sub>2</sub> + 7.9127
9.	X <sub>2</sub>	Y <sub>4</sub>	2.0198	X <sub>2</sub> + 3.5332	0.1985	X <sub>2</sub> + 6.4153
10.	X <sub>2</sub>	Y <sub>5</sub>	0.7744	X <sub>2</sub> + 1.6656	0.7480	X <sub>2</sub> + 1.8746
11.	X <sub>2</sub>	Y <sub>1</sub>	0.6885	X <sub>2</sub> + 1.6861	0.1970	X <sub>2</sub> + 5.6151
12.	X <sub>2</sub>	Y <sub>2</sub>	0.4604	X <sub>2</sub> + 3.4533	0.2999	X <sub>2</sub> + 4.3438
13.	X <sub>2</sub>	Y <sub>3</sub>	0.7928	X <sub>2</sub> + 4.1072	0.8406	X <sub>2</sub> + 4.5658
14.	X <sub>2</sub>	Y <sub>4</sub>	0.6773	X <sub>2</sub> + 1.2919	0.2378	X <sub>2</sub> + 5.2913
15.	X <sub>2</sub>	Y <sub>5</sub>	0.0959	X <sub>2</sub> + 4.1554	0.0905	X <sub>2</sub> + 3.8341
16.	Y <sub>1</sub>	Y <sub>4</sub>	0.9242	Y <sub>1</sub> + 0.0476	0.7801	Y <sub>1</sub> + 1.5579
17.	Y <sub>2</sub>	Y <sub>5</sub>	0.6719	Y <sub>2</sub> + 3.6339	0.2766	Y <sub>2</sub> + 2.6906
18.	Y <sub>4</sub>	Y <sub>5</sub>	0.9164	Y <sub>4</sub> + 4.2417	1.4871	Y <sub>4</sub> + 0.4418
19.	X <sub>1</sub> + X <sub>2</sub>	X <sub>3</sub>	-0.0948	X <sub>1</sub> X <sub>2</sub> + 9.0744	0.9633	X <sub>1</sub> X <sub>2</sub> - 4.8040
20.	X <sub>1</sub>	X <sub>2</sub>	0.2618	X <sub>1</sub> + 1.4944	0.0515	X <sub>1</sub> + 3.0654
21.	X <sub>1</sub>	X <sub>3</sub>	-0.4048	X <sub>1</sub> + 11.9760	1.1461	X <sub>1</sub> - 3.1748
22.	X <sub>2</sub>	X <sub>3</sub>	2.4964	X <sub>2</sub> - 6.4046	0.0008	X <sub>2</sub> + 7.6872

conjugate diameters of inlet were highly significant.

The transverse and vertical diameters of outlet as well as the transverse and conjugate diameters of inlet in Surti does were found to be highly significant.

#### Modification of formulae:

The actual diameters of pelvis were significantly more than the estimated diameters therefore, it was necessary to

modify the given formulae in a such way that the differences between the estimated and actual diameters became non significant. The modified formulae are presented in Table 6.

The formulae of pelvimetry recommended by Arloing (1868) and Chauveau (1891) for cattle do not appear to be applicable to the sheep and goats since the actual diameters of pelvis were significantly greater than the estimated dia-

TABLE 6. MODIFIED FORMULAE OF PELVIC DIAMETERS (cm)

	Diameters of outlet		Diameters of inlet	
	Transverse	Vertical	Transverse	Vertical
Bannur ewes	$\frac{1}{2} (A + B) + 1.25$ = D	$\frac{1}{2} (C) + 0.0$ = E	$\frac{12.2}{10} (D) + 2.43$	$\frac{13}{10} (E) + 2.70$
Surti does	$\frac{1}{2} (A + B) + 1.29$ = D	$\frac{1}{2} (C) + 1.35$ = E	$\frac{12.2}{10} (D) + 2.51$	$\frac{13}{10} (E) + 3.51$



TABLE 5. TESTING OF FORMULAE USED FOR ESTIMATION OF PELVIC DIAMETERS OF OUTLET AND INLET

(All measurements are given in cm.)

Sr. No.	Pelvic Measurements	Bannur ewes (n = 5)						Surti does (n = 9)					
		Mean	S.D.	S.E.	Range	C.V. %	T <sub>4</sub>	Mean	S.D.	S.E.	Range	C.V. %	t <sub>4</sub>
1.	Anterior width 'A'	10.64	1.46	0.65	8.3—12.0	13.75		9.41	1.22	0.41	8.1—11.3	12.93	
2.	Posterior width 'B'	4.28	0.53	0.24	3.4—4.8	12.29		3.55	0.36	0.12	2.9—3.8	10.14	
3.	Height 'C'	7.66	1.38	0.62	5.6—9.0	18.07		7.69	1.71	0.57	6.2—10.5	23.03	
<b>Estimated Diameters</b>													
<i>Outlet</i>													
4.	Transverse $\frac{1}{2}(A+B) = 'D'$	3.73	0.34	0.15	3.17—4.07	9.14		3.24	0.32	0.11	2.75—3.75	10.12	
5.	Vertical $\frac{1}{2}(C) = 'E'$	5.74	1.04	0.46	4.2—6.75	18.06		5.76	1.30	0.43	4.65—7.87	22.61	
<i>Inlet</i>													
6.	Transverse $\frac{1}{10} \frac{9.9}{10} (D)$	4.55	0.42	0.19	3.86—4.96	9.18		3.95	0.24	0.03	3.35—4.51	6.00	
7.	Conjugate $\frac{1}{10} (E)$	7.45	1.37	0.61	5.40—9.77	34.24		7.50	1.67	0.55	6.04—10.23	22.30	
<b>Actual Diameters</b>													
<i>Outlet</i>													
8.	Transverse	4.98	0.93	0.42	4.0—6.2	18.64		4.53	0.41	0.14	3.9—5.1	9.07	
9.	Vertical	6.48	1.20	0.54	4.8—8.0	18.49		7.12	0.65	0.22	6.6—8.5	9.15	
<i>Inlet</i>													
10.	Transverse	6.96	1.01	0.45	5.6—7.7	14.44		6.65	0.62	0.21	6.0—7.5	9.37	
11.	Conjugate	10.18	1.19	0.53	8.5—11.5	15.14		11.03	1.49	0.50	9.5—13.3	13.54	
<b>Differences between Actual and Estimated Diameters</b>													
<i>Outlet</i>													
12.	Transverse (8—4)	1.25	0.99	0.44	1.18—2.23	79.31	2.84*	1.29	0.40	0.13	0.7—2.05	31.24	9.60**
13.	Vertical (9—5)	0.74	0.75	0.34	—0.52—1.25	102.05	2.18NS	1.35	1.10	0.33	—0.37—2.35	75.54	3.97**
<i>Inlet</i>													
14.	Transverse (10—6)	2.43	0.91	0.41	1.03—3.54	37.27	5.93**	2.51	0.62	0.20	1.30—3.30	24.68	12.55**
15.	Conjugate (11—7)	2.70	0.53	0.24	1.83—3.20	19.65	11.25*	3.51	0.91	0.30	2.97—4.18	25.87	11.59**

Table Values: t<sub>4</sub>, 0.05 = 2.776t<sub>4</sub>, 0.01 = 4.604t<sub>8</sub>, 0.05 = 2.306t<sub>8</sub>, 0.01 = 3.355



meters and therefore, the formulae needed modifications. These formulae suggest the addition of a factor to the formulae indicated by Arloing (1868) and Chauveau (1891). Similar work was not available for comparison. From the present study it is evident that the modified formulae may give fairly accurate measurements of internal diameters of pelvis from those of external ones.

### Acknowledgement

The authors feel grateful to Dr. B. R.

Deshpande, Head of the Department of Animal Reproduction and Surgery for his continued interest and guidance. Thanks are also due to Dr. C. R. Sane formerly Professor of Gynaecology and Dr. S. M. Ajinkya, Associate Dean, Bombay Veterinary College for facilities provided during the research work. The help rendered by the Staff members of the Animal Reproduction Department is gratefully acknowledged.

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## Thyroid Status of Early Neonatal Buffalo Calves

V.M. MEHTA<sup>1</sup> and P.N. VARMAN<sup>2</sup>

### ABSTRACT

The chronological thyroid status of neonatal buffalo calves from birth to fifteen days of age was studied through the estimation of blood levels of Thyroxine ( $T_4$ ), Triiodothyronine ( $T_3$ ) and  $T_4:T_3$  ratio employing the Radio immuno assay technique. The results revealed that the new born buffalo calves maintain the peak levels of  $T_3$  and  $T_4$  in blood serum during first two days of their neonatal life. The levels of both these hormones declined gradually upto fifteen days of age, however, the decline of  $T_3$  level was much more faster than  $T_4$  level. The  $T_4:T_3$  ratio remained extremely narrow on the first two days of neonatal life and gradually got widened upto fifteen days of age. The observed peculiarity of thyroid hormone secretion in the early neonatal phase of buffalo calves can be attributed to the metabolic needs and adaptation to the extra uterine life.

\* \* \*

The influence of foetal thyroid gland on the morphogenesis and functional development of foetus is well established, (Jost, 1966). The functional status of foetal and neonatal endocrine glands of lambs and calves has been established in recent years, (Bassett and Mudtil, 1974; Nathanielsz, 1976). These glands not only modulate the metabolism of new born animal but also play a decisive role in adaptation to the hostile extra-uterine

environment as well. The young-ones with lower thyroid activities may find difficulties in adapting to newer nutritional and climatic factors and succumb to death. A recent survey in the incidence of calf mortality from birth to eight days varied between 0.6 to 3.5 per cent in Zebu cattle and 0.8 per cent in Murrah buffaloes, (Narsimha Rao, 1982). A chronological study on the thyroid status in the early neonatal life, therefore, may be useful in calf management and provide some explanation towards the metabolic and functional disorders of new born buffalo calves. Since no information is available on these lines, it was proposed to study the thyroid status of early neonatal buffalo calves through the determination of blood serum level of Thyroxine ( $T_4$ ), Triiodothyronine ( $T_3$ ) and  $T_4:T_3$  ratio right from birth to fifteen days of age.

### Materials and Methods

To study the levels of thyroid hormones, the blood samples were collected from jugular vein of healthy Murrah buffalo calves from birth to fifteen days of age. The aliquot of serum samples were stored in deep freeze ( $-10^\circ\text{C}$ ) until analysed further.

These serum samples were analysed for the concentration of Triiodothyronine ( $T_3$ ) and Thyroxine ( $T_4$ ) using Radio-

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TABLE 1. THE MEAN BLOOD SERUM LEVELS OF THYROXINE ( $T_4$ ), TRIIODOTHYRONINE ( $T_3$ ) AND RATIO OF  $T_3$  AND  $T_4$  IN NEONATAL BUFFALO CALVES FROM BIRTH TO FIFTEEN DAYS OF AGE.

Age (Days)	Serum Triiodothyronine ( $T_3$ ) (ng/ml.)	Serum Thyroxine ( $T_4$ ) ( $\mu$ g/100 ml.)	Serum $T_4:T_3$ Ratio
Birth	4.51 $\pm$ 0.25 (6)*	12.81 $\pm$ 0.06 (6)	28.43
1	5.72 $\pm$ 0.18 (6)	15.06 $\pm$ 0.86 (6)	26.32
2	3.62 $\pm$ 0.25 (6)	12.25 $\pm$ 0.73 (6)	33.84
3	2.97 $\pm$ 0.12 (4)	10.12 $\pm$ 0.50 (4)	34.07
4	2.88 $\pm$ 0.30 (6)	10.70 $\pm$ 0.39 (5)	37.15
5	2.24 $\pm$ 0.00 (1)	10.50 $\pm$ 0.31 (2)	46.35
6	n.d.	9.30 $\pm$ 0.00 (1)	—
7	1.80 $\pm$ 0.30 (6)	9.35 $\pm$ 0.10 (5)	51.94
8	1.07 $\pm$ 0.55 (5)	9.46 $\pm$ 0.70 (4)	88.41
11	1.36 $\pm$ 0.70 (6)	9.50 $\pm$ 0.90 (4)	70.44
13	0.96 $\pm$ 0.20 (8)	9.57 $\pm$ 0.51 (8)	99.68
15	0.72 $\pm$ 0.40 (6)	7.50 $\pm$ 0.72 (6)	64.66

n.d. not determined.

\* Figures in paranthesis indicate number of calves.

immuno assay technique. The kits for  $T_3$  and  $T_4$  determination were made available from M/s. Radio Chemical Centre, Amersham, England where the antigen was tagged with  $^{125}\text{I}$ . The radio activity of the samples and standards was estimated through medical spectrometer (Electronic Corporation of India). The standard curve and the estimates for  $T_3$  and  $T_4$  were obtained according to the instructions supplied with kits. The mean and the S.D. for the levels estimated were calculated following standard statistical method as described by Steel and Torri (1960).

### Results and Discussion

The mean serum levels of  $T_4$ ,  $T_3$  and  $T_4:T_3$  ratio with their standard deviations observed for the buffalo calves from birth to fifteen days of age are shown in Table-1.

The blood serum level of both  $T_3$  and

$T_4$  tended to reach at peak level from the day of birth to second day of age ( $P < 0.05$ ). Therefore, the levels of these hormones gradually but significantly declined upto fifteenth day of age except for showing an apparant ( $P > 0.05$ ) upward fluctuation in  $T_3$  level on eleventh day of age. A negative but highly significant ( $P < 0.01$ ) correlation ( $r = -0.8419$ ) between the age of buffalo calf and  $T_4$  level in blood serum was established.

The ratio between the  $T_4$  and  $T_3$  level in serum was observed to be minimum on the second day of neonatal life. Thereafter, the ratio tended to become wider upto fifteenth day of age.

The high levels of  $T_3$  and  $T_4$  in the serum of two days old buffalo calf have been observed in the cow calves of similar age, (Hernandez, et al. 1972; Mujib and Siddiqui, 1973). These observations indicate that the first two days of neonatal life of buffalo calves are crucial with



regards to metabolic and functional adaptation. Further, studies with cow calves have shown that these animals exhibit peak metabolic rate during first two days of their neonatal life (Roy, et al. 1957). It seems probable that to establish metabolism for nonshivering type of thermogenesis and to meet with sudden increase in the input of complex nutrients through colostrum, the thyroid gland activity is required to be maintained at peak and once this active phase passes off the secretory activity decline. In the in-Vitro studies on (U — C<sup>14</sup>) Glucose and (2 — C<sup>14</sup>) Acetate metabolism in the hepatic tissue of these calves, it was observed that the catabolism of both these

metabolites shoot up on the second day after birth, (Mehta and Varman, 1981). Since the changes observed in T<sub>3</sub> levels are more rapid than T<sub>4</sub> levels, the changes observed in T<sub>4</sub> and T<sub>3</sub> ratio seem to be influenced by T<sub>3</sub> more than T<sub>4</sub> levels. At present there is no explanation available for such behaviour of T<sub>3</sub> levels but it seems probable that as the age advances, the conversion of T<sub>3</sub> to T<sub>4</sub> in the thyroid gland become more efficient.

The present studies have shown to believe that the peak activity of thyroid glands in the early neonatal period is essential in the physiological adaptation to the extra-uterine life.

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## Studies on Post Partum Anoestrous Condition in Surti Buffaloes with Relation to Season and Body Weight and Trial with 'Prajana'<sup>1</sup>

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Anoestrous condition influences the economics of milk production. Rao and Murthy (1971) and Kodagali (1978) studied the effect of season and body weight on reproductive performance. Prajana helps in inducing oestrus and ovulation (Porwal *et al.*, 1976, Kodagali *et al.*, 1980).

The present work was undertaken to study the effect of season and body weight and to assess the efficacy of "Prajana" in induction of oestrus and fertile oestrus.

### Materials and Method

#### Clinical trials:

A total of 64 buffaloes of Surti breed with true anoestrous condition were selected for the study. Out of which 56 buffaloes were included in the treatment trials with Prajana capsules (orally) and Prajana injections (I/m) and 8 buffaloes were kept in control group.

#### Season:

The work was carried out during the period of one full year. The year was divided into two seasons for experimental purpose viz., Low breeding season (March to August) and high breeding season (September to February).

#### Body weight:

To study the influence of body weight, the length and girth measurements of all the animals were measured and the body weights were calculated using Shaeffer's formula as cited by Sastry and Thomas (1976).

$$\text{Live wt. (in lbs)} = \frac{L \times G^2}{300}$$

where,

L = Length in inches shoulder to pin bone,

G = Girth circumference in inches.

### Results and Discussion

The overall results in treatment group (with Prajana capsules and injections) vs Control group have been presented in Table-1.

Statistically the treatments have been proved to have significant effect on oestrus induction. The results on oestrus induction and fertile oestrus induction percentage nearly agree with the results reported by Galhotra *et al.* (1970), Kodagali *et al.* (1973), Porwal *et al.* (1976) and Deshpande and Sane (1977).

The comparison of results with Prajana injection, and capsule group have been presented in Table-2.

<sup>1</sup> An indigenous drug of Indian Herbs Research and Supply Co., Saharanpur, (U.P.).  
Part of M.V.Sc. studies.



TABLE 1. RESULTS OF PRAJANA TRIALS IN TREATMENT VS CONTROL GROUP

Sr. No.	Item	Treatment group		Control group	
		No.	%	No.	%
1	No. of buffaloes in trial	56		8	
2	No. of oestrus induced/occurred	46	82.14	4	50.00
3	No. conceived	27	48.21	—	—
4	Oestrus induction/occurrence interval in days	15.48 ± 3.35		31.25 ± 2.90	
5	Fertile oestrus interval (days)	31.95 ± 9.14		—	

TABLE 2. RESULTS OF PRAJANA INJECTION VS CAPSULE GROUP

Sr. No.	Item	Prajana No.	inj. %	Prajana No.	cap. %
1	No. of buffaloes	37		19	
2	No. oestrus induced	30	81.00	16	84.20
3	No. conceived	19	51.30	8	42.10
4	Oestrus induction interval (days)	15.22 ± 2.70		15.75 ± 4.48	
5	Fertile oestrus interval (days)	30.00 ± 10.55		38.91 ± 7.68	

TABLE 3. RESULTS OF PRAJANA TRIALS IN LOW BREEDING SEASON VS HIGH BREEDING SEASON.

Sr. No.	Item	No.	L. B. S. %	No.	H. B. S. %
1	No. of buffaloes	25		31	
2	No. oestrus induced	20	80.00	26	83.80
3	No. conceived	10	40.00	17	54.80
4	Oestrus induction interval (days)	15.64 ± 3.51		15.33 ± 3.43	
5	Fertile oestrus interval (days)	30.16 ± 11.36		33.37 ± 6.42	

Statistically there was no significant difference in the results between Prajana injection and capsule group for the number of oestrus and fertile oestrus inductions.

The results obtained in Low breeding season (L.B.S.) vs High breeding season (H.B.S.) have been illustrated in Table-3.

Apparently the results in high breeding season were better but statistically there

was no significant difference between the results of low breeding season and high breeding season. Similar observations were made by Rao (1976) and El foulty *et al.* (1977).

The relation of body weights with oestrus induction and fertile oestrus induction in buffaloes have been presented in Table-4.

Statistically, the body weight had signi-



TABLE 4. BODY WEIGHTS AND OESTRUS &amp; FERTILE OESTRUS INDUCTION IN BUFFALOES.

Body weights (kg)		250—300		300—350		350—400		400 & above	
		No.	%	No.	%	No.	%	No.	%
Oestrus induction	Yes	3	37.50	16	78.26	21	91.30	8	80.00
	No	5	62.50	5	21.74	2	8.70	2	20.00
	Total	8	100.00	23	100.00	23	100.00	10	100.00
Fertile oestrus	Yes	1	12.50	12	52.17	9	39.13	5	50.00
	No	7	87.50	11	47.83	14	60.87	5	50.00
	Total	8	100.00	23	100.00	23	100.00	10	100.00

ficant effect on oestrus & fertile oestrus induction. As the body weight increased, the percentages of oestrus and fertile oestrus were in increasing order. King (1968), Patil (1976) & Kodagali (1978) have reported higher C. R. with increase in body weight. Thus the results under present study agree with the results already reported and compared.

### Summary

The present study reported on the trials of Prajana injections & capsules for treatment of anoestrous buffaloes is on the effect of season and body weight in

induction of oestrus and fertile oestrus.

With Prajana treatment oestrus was induced in 82.14 per cent animals with the mean oestrus induction interval of 15.48 days and 48.21 per cent animals conceived within the average period of 31.25 days.

There is no significant difference between low breeding and high breeding season for oestrus and fertile oestrus induction.

Body weight has been found to be related with oestrus and fertile oestrus inductions.

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## Retention of Fetal Membranes in Buffaloes-Serum Proteins and Blood Glucose Levels

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### ABSTRACT

The serum total proteins, albumin, globulin A/G ratio and blood glucose were estimated in 8 buffaloes retaining fetal membranes (FM) and 25 buffaloes without FM retention. The mean levels of serum total proteins, albumin, globulin and the A/G ratio on 285th day of gestation were  $7.55 \pm 0.51$ ;  $3.30 \pm 0.20$ ;  $4.25 \pm 0.33$  gm per cent and  $0.83 \pm 0.23$ , respectively in buffaloes not retaining FM. These values were higher than those in buffaloes retaining FM on corresponding days, but the differences were not statistically significant. In both, the animals retaining or not retaining FM, the levels of serum total proteins, albumin and globulin continued to fall from 285th day of gestation to 12 hours post-partum and then again started rising till they reached the pre-partum levels by day 10th post-partum.

The blood glucose levels were found to be  $52.66 \pm 1.84$  and  $50.67 \pm 0.93$  mg per cent on 295th day of gestation in buffaloes not retaining and retaining FM, respectively. In both the groups of animals the blood glucose levels increased at 12 hours post-partum and then fell down from 12 hours after parturition till 10th day post-partum.

\* \* \*

The data available in literature on serum proteins and blood glucose levels

during various physiological and disease conditions in buffaloes is very scanty. Nawaz and Siddiqui (1974) did not find any significant variation in the protein levels in buffaloes during various physiological conditions, whereas, Pandit (1978) and Seshagiri *et al.* (1979) have reported physiological variations in serum protein levels in buffaloes in relation to pregnancy and puer-perium. The blood glucose levels have been reported lower in post-partum anestrus buffaloes than in normal cycling buffaloes (Dhoble, 1978). The present study was undertaken to find out the serum proteins and blood glucose levels in buffaloes retaining FM.

### Materials and Methods

The studies were conducted on 33 buffaloes of Murrah and Nili Ravi breeds. Blood samples were collected from 8 buffaloes retaining FM and 25 buffaloes without FM retention on 285th, 295th and 300th day of gestation and 12th hour, 1st, 2nd, 3rd, 5th and 10th days post-partum. The buffaloes not dropping their FM within 12 hours post-partum were considered to have retained FM. The animals were in their 1st to 6th lactation during the study. All the animals were maintained under standard managerial conditions.

The blood (3-4 ml) was collected in sodium fluoride bulbs and kept at 4°C



TABLE 1. SERUM TOTAL PROTEINS, ALBUMIN (A), GLOBULIN (G) AND A/G RATIO IN BUFFALOES NOT RETAINING AND RETAINING FM.

Constituents	Groups	Gestation days		12th hour postpartum	Days post-partum		
		285th	300th		3	5	10
Serum total protein (gm%)	Not retaining FM	7.55±0.51	7.06±0.35	6.25±0.33	6.48±0.35	6.91±0.37	6.98±0.43
	Retaining FM	6.71±0.61	6.25±0.67	5.03±0.75	5.13±0.55	6.00±0.62	6.70±0.69
Serum albumin (gm %)	Not retaining FM	3.30±0.20	2.96±0.15	3.16±0.17	3.29±0.19	3.70±0.22	3.79±0.30
	Retaining FM	3.11±0.28	2.70±0.41	2.25±0.37	2.62±0.26	3.09±0.30	3.54±0.35
Serum globulin (gm %)	Not retaining FM	4.25±0.33	4.10±0.23	3.09±0.23	3.19±0.21	3.21±0.21	3.19±0.18
	Retaining FM	3.60±0.34	3.55±0.48	2.78±0.39	2.51±0.32	2.91±0.33	3.16±0.37
A/G ratio	Not retaining FM	0.83±0.23	0.74±0.19	1.10±0.40	1.79±0.35	1.21±0.40	1.20±0.42
	Retaining FM	0.87±0.08	0.75±0.08	0.80±0.18	1.10±0.27	1.09±0.16	1.18±0.29

temperature for the estimation of glucose. The glucose was estimated in whole blood using the method of Folin and Wu (1920). For the estimation of total proteins, albumin and globulin, the blood was allowed to clot and serum was separated by centrifugation. The serum samples were stored at -20°C temperature, until used for estimations. Serum total protein, albumin and globulins were estimated by the biuret method (Varley, 1967).

### Results

The serum total proteins, albumin and globulin levels and A/G ratio on 285th and 300th days of gestation, and 12th hour, 3rd, 5th and 10th days post-partum in buffaloes with and without retention of FM is shown in Table 1. In buffaloes not retaining the FM, the serum total proteins, albumin and globulin levels were higher during late gestation. These levels continued to decline as the parturition approached and again returned to the pre-partum levels by 10th day post-partum. In buffaloes retaining FM beyond 12 hours of parturi-

tion, the levels of serum total proteins, albumin and globulin followed similar pattern as that of buffaloes not retaining FM. However, the levels of all the three constituents were lower in the later group. The A/G ratio was found to be inconsistent during the period of study.

The blood glucose levels on 295th day of gestation, and 12th hour, 1st, 2nd, 3rd and 10th days post-partum in buffaloes with and without retention of FM are shown in Table 2. The blood glucose in buffaloes was high during late gestation and fell from 1st day through 10th day

TABLE 2. BLOOD GLUCOSE (mg %) IN BUFFALOES NOT RETAINING AND RETAINING FM.

Days	Not retaining FM	Retaining FM
295th day of gestation	52.66±1.84	50.67±0.93
12th hour post-partum	61.45±1.68	56.13±3.49
1 day post-partum	59.60±1.56	55.24±3.23
2 day post-partum	56.85±1.74	53.02±2.59
3 day post-partum	55.79±1.87	53.62±3.32
10 day post-partum	53.74±1.35	48.59±2.25



post-partum. The glucose levels were higher in buffaloes not retaining FM than in buffaloes retaining FM on all the days of observation. However, the difference was significant only on 10th day post-partum ( $P < 0.05$ ).

### Discussion

The values of serum total proteins were lower in post-partum buffaloes than in buffaloes during advance pregnancy. Nawaz and Siddiqui (1974) did not find any significant variation in serum total proteins in buffaloes under various physiological conditions. Similar trend was also observed in the levels of serum albumin and globulin. The levels of all the three serum constituents were lower in buffaloes retaining FM. Although the differences were not statistically significant, a consistent low levels of all the three constituents throughout the study period is worth attention.

The blood glucose levels were higher in buffaloes not retaining FM on all the days of sampling. However, only on 10th day post-partum the differences

were statistically significant ( $P < 0.05$ ). The lower magnitude of difference in the blood glucose levels of buffaloes retaining and not retaining FM as compared to that in the cows (Dutta and Dugwekar, 1983) may be due to species differences. Boitor *et al.* (1972) reported that in addition to glucose, serum calcium, phosphorus and magnesium levels were also low in cows with retained FM. It is possible that in buffaloes also glucose levels estimated along with other parameters during late gestation might help in predicting post-partum complications like retained FM.

### Acknowledgements

The authors are thankful to Dr. R.D. Sharma, Professor-cum-Head, Department of Obstetrics and Gynaecology, College of Veterinary Science, Punjab Agricultural University, Ludhiana for providing the research facilities. The financial assistance provided to the first author in the form of Junior Research Fellowship by the Council of Scientific and Industrial Research, New Delhi is thankfully acknowledged.

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## Freemartinism in a Crossbred Heifer

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A freemartin is a sexually imperfect, usually sterile, female partner of a pair of heterosexual bovine twins, in which the development of the gonads has been controlled by the inter-circulatory system of the male and female twins (Hafez and Jainudeen, 1966). About 92 per cent of such females are sterile.

The present report, describes the anatomical, histological findings and the chromosomal pattern and plasma estradiol concentration in a Jersey-Holstein-Friesian cross freemartin.

### Materials and Methods

A two and a half year old Jersey-Holstein-Friesian heifer of the HAU, animal farm was reported to be anoestrus. The animal was a heterosexual twin, with a birth weight of 22 kg.

#### *Clinical findings:*

The external genitalia were apparently normaly, except a small vulva. Rectal palpation revealed a short vagina, the cervix was not palpable and chord like uterine horns. At the base of the uterus, on either side, two glandular structures resembling seminal vesicles were palpable. The gonads could not be palpated on clinical examination. The vagina was non-patent ending in a blind pouch. The animal was sacrificed and the genitalia

dissected out for examination:

#### *Histopathology:*

The seminal vesicles, vas deferens, uterine horns and the rudimentary gonads were processed routinely for histopathology.

#### *Karyotyping:*

Chromosome preparations were made from whole blood cultures using TC 199 medium (Disco), supplemented with amino acids and calf serum (Halnan, 1977). The air dried preparations were stained with carbol fuchsin. One hundred, well spread, metaphases were observed to study the chromosome number and morphology and the Karyotype prepared.

#### *Estradiol assay:*

Plasma estradiol concentration was assayed following the method of Abraham (1975) using a kit from Hypolab (Switzerland).

### Results

Phenotypically the animal had the general appearance of a female (Fig. 1). However, the udder was poorly developed. A small tuft of hair was present at the lower vulval commissure. The distance from the vulvar lips to the blind vaginal pouch was 10.7 cm (Fig. 2). The two seminal vesicles were present at a distance of 14 and 16 cms. from the vulvar opening. The distance between the vulva and the uterine horns was 17 cms. The gonads were very small.

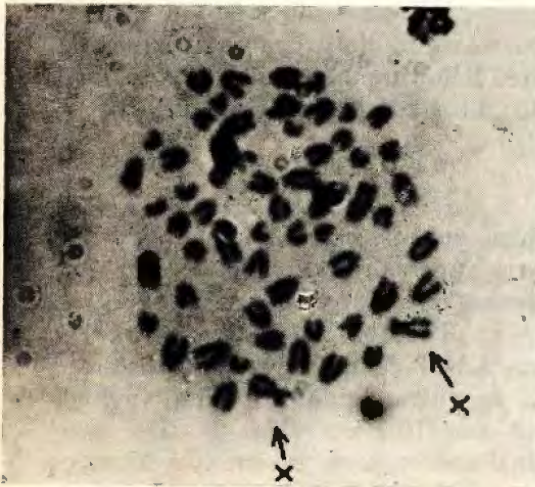




Jersey-holstein-friesian freemartin heifer



Internal Genitalia of the freemartin heifer showing a Tuft of hair on the lower vulval commissure and seminal vesicles (1+2) uterine horns, unseparated (3), ureters (4) and urinary bladder (5)



Karyotype showing  $\times \times$  chromosomes



Karyotype showing X Y chromosomes



The chromosomal number was observed to be sixty. From the one hundred metaphases studied, two thirds showed XX and the remaining XY sex chromosomes. Thus the animal had a 60 XX/XY chromosomal complement. (Fig. 3 and 4).

Histologically the gonads resembled completely hypoplastic ovaries, with the ovarian stroma comprising of thick connective tissue layer of tunica albuginea. Some anovulatory cords and follicles were observed. No structures resembling seminiferous tubules were observed. The lobulated structures on either side of the uterine horns resembled the seminal vesicles.

The peripheral plasma estradiol-17 beta concentration was below the detectable limits of the assay, which was about 4 pg./ml.

### Discussion

Anoestrous is the major symptom of freemartinism (Arthur, 1964, Roberts, 1971). Although the animal under report was also anoestrous, the possibility of normal fertility in such animals cannot be excluded (Miyake et al; 1980).

The length of the vulvo-vagina in the case under report was 10.7 cm. Kastli (1974) observed the vaginal length in bovine freemartins to vary between 4.5 and 7.5 cm. The non patency of the vagina observed in this animal has also been reported by Ficher (1946) and Kangawa et al; (1965). The white tuft of hair on the lower vulvar commissure has also been described by Swett et al (1940). Possibly hypertrophy of the clitoris (Kangawa et al (1965), Moore, 1966) may be observed in animals with gonads resembling ovo-testes, whereas failure of the clitoris to hypertrophy, seen in the present case, is observed in

animals with gonads resembling hypoplastic ovaries.

Considerable variations, from complete suppression of the uterine development to the presence of rudimentary horns (Goss, 1950) is observed in freemartins. In the present case the rudimentary horns were thread like and lacked patency. The presence of the seminal vesicles corresponds with the findings of Short et. al; (1969) and Laster et al (1971). The hypoplastic condition of the ovaries has also been reported by Roberts (1971).

The 60 XX/XY chromosomal complement is in agreement with the findings of Dunn et al; (1968) and Wilkes et al; (1981). Sex chromosomal chimerism has been reported both in male and female cotwins (Ohno et al; 1962). The excess of one cell type in both members of a set of heterosexual twins has been reported by Basrur and Stolz (1966).

The anastomosis of the chorionic vessels preceeds the migration of the primordial sex cells from the yolk sac to the genital ridge in the freemartins. Thus the migratory primordial germ cells pass from the male foetus to the female foetus in early pregnancy leading to arrest or defective development of the female genital system (Ohno et al; 1963). However, the first signs of freemartinism may be independent of the XX/XY chimerism. Consequently, although chromosomal abnormalities alone may not be responsible, yet cytogenetic studies can be useful for diagnostic purposes.

The undetectable levels of plasma estradiol-17 beta concentrations observed in the present animal are more or less expected in view of the completely hypoplastic ovaries. Similar observations have been reported by Short et al; (1969).



## Summary

The clinical findings, anatomical and histological features of the genital tract of a 2½ year old Jersey-Holstein-Friesian cross heterosexual cotwin heifer which failed to exhibit oestrous are described. The vagina was small and ended in a blind sac, the cervix was absent and uterine horns small and rudimentary. The

gonads resembled hypoplastic ovaries and two lobulated structures, histologically similar to seminal vesicles were present on either side of the rudimentary uterine horns. The animal had a 60 XX/XY chromosomal complement, with about two-third cells showing XX and the rest XY. The plasma estradiol concentration was below the detectable limit of 4 pg/ml.

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## Genital Infections and Treatment in Surti Buffaloes

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### ABSTRACT

The present study was undertaken with the objectives of knowing the micro-organisms from the cervicovaginal mucus and their antibiotic sensitivity in-vitro and to find out the effect of treatment in-vivo for better conception. For the study, 87 Surti buffaloes were chosen randomly. The cervico-vaginal mucus samples were collected and subjected to cultural isolation and identification of genital microflora as well as antibiotic sensitivity test, 26.44% animals had no genital infection. The isolates found were: 29.83% gram negative bacilli organisms, 31.57% gram positive bacilli organisms without spore, 7.91% gram positive bacilli organisms with spore and 30.69% gram positive cocci organisms. The sensitivity of antibiotics to the isolates were: Penicillin - 73.85%, Streptomycin-70.77%, Tetracycline -75.38%, Ampicillin -58.46%, Neomycin -95.38%, Furadentin -69.23% and Gentamycin -87.69%. The animals were treated with the antibiotics for which specific sensitivity was observed and on resuming normalcy they were inseminated and followed for pregnancy. At the close of study, out of 87 buffaloes, 66 (75.86%) conceived.

\* \* \*

Infection plays a major role as a single cause in repeating the oestrous cycle. This is brought out by early death of zygote or embryo (Roberts, 1971).

Each repeated oestrous cycle is reflected in the form of decreased calf crop and reduced milk production during the life-time of the animal resulting into economical loss to the farmers (Hafez, 1968). Narsinh Rao and Keshav Murthy (1971) have reported that 32.28% buffaloes had reproductive failure due to non-specific infections. Narsinh Rao and Keshav Murthy (1972) reported 19.41% of the infertility cases due to endometritis. Chauhan and Meher Singh (1979) reported that 26.60% cases of anoestrus buffaloes had infection of genital tract. In the cattle infertility scheme report (1981-82) it is stated that 16.00% of infertility problems were due to endometritis.

Treatment to combat genital infection with different antibiotics are under trials. In the present study attempts were made to study the genital microflora and to determine the therapeutic efficacy of different antibiotics.

### Materials & Methods

For the study, 87 Surti buffaloes were chosen randomly. The genital discharges were utilised for bacteriological investigation and on culturing for in-vitro sensitivity tests. The samples were collected aseptically by aspiration using sterilized glass pipette (10 ml cap.), the pointed end of which was connected to a syringe with rubber junction by recto-vaginal technique (Pattabhiraman *et al.*, 1967;



TABLE 1. ISOLATION AND IDENTIFICATION OF ORGANISMS FROM THE CERVICOVAGINAL MUCUS.

Sr. No.	Organisms	No. isolated	Percent
1.	Samples positive	65	73.56
2.	Samples negative	22	26.44
3.	<i>Gram negative bacilli</i>		
	<i>Escherichia coli</i>	11	9.65
	<i>Enterobacter hafnac</i>	1	0.88
	<i>Enterobacter cloacae</i>	1	0.88
	<i>Profeus morganil</i>	2	1.75
	<i>Pasturella hemolytica</i>	1	0.88
	Unidentified gram-ve bacilli	18	15.79
4.	<i>Gram positive bacilli without spore</i>		
	<i>Coryn. pyogenes</i>	6	5.26
	<i>Coryn. murium</i>	11	9.65
	<i>Coryn. ulcerans</i>	3	2.63
	<i>Coryn. egni</i>	6	5.26
	<i>Coryn. xerosis</i>	3	5.26
	<i>Coryn. ovis</i>	3	2.63
	<i>Kurthia</i>	1	0.88
	<i>Listeria monocytogenes</i>	3	2.63
5.	<i>Gram positive bacilli with spore</i>		
	<i>Bacillus coagulans</i>	5	4.39
	<i>Bacillus polymyxa</i>	1	0.88
	<i>Bacillus murcerans</i>	1	0.88
	<i>Bacillus subtilis</i>	1	0.88
	<i>Bacillus circulans</i>	1	0.88
	<i>Gram positive cocci</i>		
	<i>Stah aureus</i>	10	8.77
	<i>Staph epidermidis</i>	14	12.28
	<i>Micrococcus</i>	6	5.26
	<i>Strepto fecalis</i>	2	1.75
	<i>Strepto lactis</i>	2	1.75
	<i>Strepto mitis</i>	1	0.88

TABLE 2. DETAILS OF RESULTS OF ANTIBIOTIC SENSITIVITY TESTS

Sr. No.	Antibiotics	No. of cases Sensitive to antibiotic	Percentage of cases sensitive to antibiotic
1.	Penicillin	48	73.85
2.	Streptomycin	46	70.77
3.	Tetracyclin	49	75.38
4.	Ampicillin	38	58.46
5.	Neomycin	62	95.38
6.	Furadentin	45	69.23
7.	Gentamycin	57	87.69

Panangala *et al.*, 1978). After collection, the samples were sent to Bacteriology laboratory and processed immediately.

Isolation work was done as per Cruickshank (1965). The pure isolates were subjected to in-vitro antibiotic sensitivity test as per the method recommended by Bauer *et al.* (1966). Antibiotic discs supplied by Pasteur Biological Laboratories (India) were used. The results were interpreted as per the chart furnished by them. The isolates were tested for their sensitivity to seven commonly used antibiotics viz., Penicillin, Streptomycin, Tetracycline, Ampicillin, Neomycin, Furadentin and gentamycin.

Depending upon the in-vitro sensitivity results, the animals were treated by effective antibiotics administered by intra-uterine route. These animals were further periodically examined. Those animals which came into normal oestrus were inseminated. These were further followed up for pregnancy diagnosis after 6-8 weeks of insemination by rectal palpation (Zemjanis, 1962).

## Results and Discussion

In the cultural isolation work the organisms found were of single type, two types or three types from the same animal or the samples were negative for any infection. In all, out of 87 animals under study, 22 (26.44%) animals had no genital infection. The frequency of occurrence of different species of bacteria in various groups of animals have been shown in the Table-1. Prasad and Mallick (1966), Narsinh Rao and Keshav Murthy (1971, 72), Sarov and Dimitrov (1973), Krishna Murthy (1974), Verma and Tyagi (1974), Sinha *et al.* (1977), Chauhan and Meher Singh (1979), Kodagali *et al.* (1980), Derasahri *et al.*



(1981) and Kharde and Patil-Kulkarni (1981) have reported on the occurrence of micro-organisms from the cervico-vaginal discharges of cows and buffaloes.

The results of antibiotic sensitivity tests have been detailed in Table-2. Savov and Dimitrov (1973), Myuirsepp *et al.* (1975), Daniels *et al.* (1976), Sinha *et al.* (1976), Kodagali *et al.* (1977), Sinha *et al.* (1977), Suryanarayan Murthy and Narsinh Rao (1979), Kodagali *et al.* (1980) and Derashri *et al.* (1981) have reported on different antibiotics effective against various genital microflora and the results of their treatment.

Under the present study the animals were treated with the antibiotics for which the sensitivity was observed. On

resuming normalcy they were inseminated and were further followed up for pregnancy diagnosis. At the close of study, out of 87 Surti buffaloes 66 (75.86%) buffaloes conceived.

It is suggested that for better conception rates in repeating animals it is necessary to have the cultural isolation of genital discharges and their antibiotic sensitivity test and then only treatment with the effective antibiotic should be undertaken.

### Acknowledgement

Thanks are due to authorities of Gujarat Agricultural University, Anand Campus Anand for providing the necessary facilities for the study.

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## Studies on the Incidence of Infertility in Crossbreed Cattle in Hassan District of Karnataka

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### ABSTRACT

Incidence of infertility conditions were investigated in 1975 apparently healthy crossbred cattle in Hassan district of Karnataka State. Non infectious cause were about 67.14% compared to 32.86% of infectious causes. The percentage of incidence of various reproductive disorders were 24, 20, 18.18, 17.97, 3.25, 2.84 and 0.66 respectively for anoestrus, underdeveloped genitalia, repeat breeder, persistent corpus luteum and cystic ovaries among the functional disorders, while, the percentage of incidence of infectious disorders were 27.39, 2.43, 2.38 and 0.66 respectively for cervicitis, metritis, granular vaginitis and oopharitis. In the above two groups anoestrus and cervicitis were more common. Season and month were found to have significant influence on the occurrence of infertility.

\* \* \*

The incidence of infertility in cross breed cattle is very high and wide spread, for reasons of clinical or non clinical pathological conditions of genitalia, or lack of timely insemination with quality semen, Roberts (1971).

Infertility in cross breeds may be due to (1) Infectious diseases caused by the various agents, like bacteria and viruses (2) Hormonal imbalance, (3) Nutritional causes and (4) Congenital and hereditary factors. Kodagali (1968, 1975) observed high percentage of anoestrus and repeat breeding in Gir cows. Dessouky *et al* (1969) reported 23% and 17% inactive ovaries in indigenous and cross breed cows respectively. Published reports on the incidence, nature and magnitude of prevalence of these conditions however appeared to be scanty. The present study was taken up to assess the magnitude and nature of incidence of infertility problems in cross breed cattle population of Hassan district of Karnataka.

### Materials and Methods

One thousand nine hundred and seventy five healthy cross breed cattle, owned by the private cattle breeders in Alur, Belur, Arakalgud, Channarayaptana, Holarasipura and Sakleshpur taluks and in and around Hassan districts of Karnataka state, formed the material for the study. Majority of the cows in the taluks of Hassan district are of Jersey and Holstein Friesian. In general cows are looked after

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TABLE 1. ANALYSIS OF VARIANCE SHOWING THE EFFECTS OF MONTHS, SEASONS AND DISORDERS OF INFERTILITY IN COWS

Source of Variation	d.f.	S.S.	m.s.	F. Value
Between Months	11	43811.09	3982.83	6.61*
Between Seasons	2	38185.04	16592.52	27.55**
Between Disorders	9	11140.32	1237.81	2.06 N.S.
Error	87	52389.34	602.18	

\* Significant at 5% level

\*\* Significant at 1% level

ns: Non significant.

by women folk and managed and feed under the stalfed conditions. The cows are housed in a locally constructed barns. The study was conducted from January 1981 to December 1981. The animals were reportedly subjected to detail Gynaecological examination for various reproductive disorders, during infertility camps conducted jointly by the Karnataka Dairy Development corporation, Hassan Unit and Diagnostic laboratory of University of Agricultural Sciences, Hassan. The several disorders recognised were categorised into two main groups. 1. Functional form of infertility which included disorders like Anestrus, repeat-breeder, persistent corpus luteum (PCL) cystic ovary and small genitalia, and 2. Infectious type which included cervicitis, metritis, pyometra, grannular vaginitis and oopharitis.

The data were arranged monthwise, and the year was divided into three seasons, Viz Winter (November-February), Summer (March-June) and Rainy (July-October) to see the effect of climatic conditions on the incidence of infertility. Monthwise average value was calculated for each disorder and its percentage in terms of total cases was determined. Analysis of variance viz: Snedecor and Cochran (1968) was made to see the

effect of months seasons, and reproductive disorders on the incidence of infertility. Cases found significant in the analysis of variance put to critical difference test.

### Results and Discussion

A total of one thousand nine hundred and seventy five (1975) cross breed cows under field conditions were studied in Hassan District of Karnataka. These cross breed cows were brought for detail gynaecological examination for various reproductive disorders during infertility camps. Functional form of infertility included Anoestusum, Repeat breeding, persistent corprluteum (PCL), cystic ovary and atrophic or small genitalia, and Infectious type namely cervicites, metritis, pyometra, grannular vaginitis and oopharitis were included. The average monthwise value for type of the disorder is presented. The incidence of cervicitis was the highest 27.39% followed by anestrus 24.20% and small genitalia 17.97%. Cases of cystic ovary were far less frequent.

Analysis of variance was made to see the variations due to months, seasons, and disorders were presented in Table I. From this table it was observed that the effect of months and seasons were significant on the incidence of infertility.



TABLE 2. CRITICAL DIFFERENCE BETWEEN VARIOUS DISORDERS

Particulars	Cystic ovary	Oophoritis	Pyometra	Metritis	PCL	Repeat Breeder	Small genitalia	Functional ovary	Non functional ovary	Cervicitis
Disorders means C.D.	F	J	I	H	D	C	F	A	B	G
@ 0.05 + 19.94	1.08	1.08	3.92	4.00	4.67	5.42	29.58	29.92	39.83	45.08

Disorders E, J, I, H, D and C are same and they are significantly different with any one of the F, A, B and G in another Group.

This indicated that climatic conditions have significant influence on the level of infertility in cows. But the variations due to disorders was significant at 5% level. Critical difference were calculated to ascertain the roll of individual abnormality on the causation of infertility. From the table II it was seen that cervicitis was the major cause 45.08% under infectious nature of infertility among cross breed cows, and its incidence was significantly higher compared to non-functional ovary 39.83%. Kodagali (1968) observed high percentage of anestrus in Gir cows and heifers. In another study Kodagali (1975) reported 47.89% anestrus in Gir cows. From this study we observed that the relative high incidence of anoestrus may be due to lower nutritional value, genetic constitution of the animal and improper detection of heat particularly among the silent heat breeders. These observations were compared with the results of Dessouky and Juma (1973) who found 10.75% cases of repeat breeding in cows. The incidence of small genitalia 29.58% also differed significantly with other disorders. Dessouky and Juma (1973) observed the possible causes of high incidences of the disorder might be due to low nutritional level, delayed ovulation non fertilization ovum due to untimely insemination, poor quality of semen and defect in the genital tract.

The incidence of small genitalia was 17.97% and anestrus due to non functional ovaries was 18.18% which was mostly caused by the low nutritional level. Dessouky *et al.* (1989) reported 23.00% and 17.00% inactive ovaries in native and cross breed cows respectively. The cases of persistent corpus luteum and cystic ovary were comparatively less 2.84 and 0.66% respectively. In the present study under report the infectious form of infertility included cases of cervicitis, metritis, pyometra, and granular vaginitis. Out of these cervicitis was more common 29.39% followed by metritis 2.43%. The cases of functional infertility were roughly double times more frequent 67.14% than infectious ones 38.86%. It is evident from the Table I that the effect of month and seasons were significant and highly significant on the incidence of infertility. This clearly indicates that the climatic conditions have significant influence on the level of fertility in cows. However, that variations due to disorders was non significant. Critical differences were calculated to ascertain the role of individual abnormality on the causation of infertility. From the table II the disorders viz. cystic ovary, pyometra, oophoritis, metritis, persistent corpus luteum (PCL) and repeat breeder are same and they are significantly different with any one of the small genitalia,



functional ovary, nonfunctional ovary, cervicitis in another group.

### **Acknowledgements**

The authors wish to thank the authori-

ties of Karnataka Dairy Development corporation, Hassan Union, Hassan for all the assistance and help.

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## Early Pregnancy Diagnosis in Cattle

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### ABSTRACT

A study was carried out to compare the efficiency of the barium chloride test and milk copper sulphate coagulation test used for early pregnancy diagnosis in cattle. In all 215 cows and heifers were tested at different stages of pregnancy after breeding. The results of tests were confirmed by rectal examination after 60 days of breeding.

#### *Barium chloride test with Urine:*

Urine samples from 215 cows and heifers were subjected to barium chloride test. The test gave 64.84, 92.18 and 83.59% accuracy at 15 to 25, 26 to 35 and 36 to 45 days of pregnancy respectively. The test results from 87 non pregnant animals were 94.5% accurate. The test was found to be more accurate in the III parity Group and during 26 to 35 days of pregnancy.

#### *Milk copper sulphate coagulation test:*

Milk samples from 163 cows were tested. The test gave 52.88, 64.42 and 75.96% accuracy at 15 to 35, 26 to 25 and 36 to 45 days of pregnancy respectively. The test results from 59 non-pregnant cows were 62.55% accurate.

Overall comparison of results of two tests, the barium chloride test was found to be more accurate, simple and easy to perform.

\* \* \*

A reliable method of early pregnancy diagnosis can be of great economic value to the dairy cattle breeders. The early identification of non-pregnant animals would help in closely observing them for oestrous and were necessary they could be treated promptly for infertility. 15 to 25 per cent of all cows and heifers failing to exhibit oestrus after breeding are actually nonpregnant (Zemjanis, 1970). When it is recognised that every missed oestrus means a loss of approximately 3 weeks production, the significance of timely detection of the pregnant and particularly the non-pregnant, animals is extremely important. Therefore, an attempt was made to evaluate the efficiency of Barium-chloride test and Milk copper Sulphate coagulation test for early pregnancy diagnosis in cattle.

### Material and Methods

The present study was conducted at the dairy farm attached to the college of Veterinary and Animal Science, Bikaner possessing 56 Rathi and crossbred cows and at the Nagur cattle Breeding Farm, locate in Nagur District, possessing 101 Naguri animals. Other animals brought to Artificial Insemination Centre of the college for insemination purpose were also included. Thus a total of 215 cows and heifers formed the material for present study. The tests were performed by using urine and milk for detection of early pregnancy.



#### *Barium chloride test with urine:*

The urine samples from 215 cows and heifers were tested with barium chloride solution between 15 to 25, 26 to 35 and 36 to 45 days after breeding. Urine sample collected in a clean dry test tube was subjected to the barium chloride test. The method described by Temblador and Land (1971) was followed. 1 per cent solution of barium chloride (Analar) was prepared in distilled water. In a dry clean test tube approximately 2 to 3 ml urine was taken and equal volume of 1 per cent barium chloride solution was added. If the urine showed white precipitation of varying degrees the test was negative animal was diagnosed nonpregnant. If the urine sample remained clear, the animal was diagnosed pregnant.

#### *Milk Copper sulphate coagulation test:*

Milk samples were collected between 15 to 25, 6 to 35 and 36 to 45 days after breeding from each animal 10 ml of 3% copper sulphate solution was placed into a 20 ml test tube. Then 1 ml of milk was added into test tube and stirred for few minute. If the milk coagulated, the test was positive for pregnancy. If the mixture remained homogenous the cows were considered nonpregnant (Abilay and Roussel, 1975).

All the cows and heifers included in present study were tested for confirmation of pregnancy after 60 days of breeding by rectal examination as described by Zemjanis (1970). The results obtained through the rectal confirmation were compared with those obtained by afore said pregnancy diagnosis to know the efficiency of the two tests.

### **Results and Discussion**

The urine samples from 215 cows and

heifers were tested with barium chloride solution.

The percentage of accuracy of barium chloride test recorded by Maslov and Smirnow (1965) was 95 to 100% between 15 to 210 days of pregnancy. Temblador and Landa (1971) reported 88.7 and 99.4 per cent accuracy of beef and dairy cows respectively after 15 days of breeding. While in present study 79.98 per cent of accuracy was recorded between 15 to 45 days of pregnancy in cattle.

However, the barium chloride test with urine is an easiest chemical test with fair accuracy for pregnancy detection. The progestational influence probably lowers sulphate excretion in the urine. When 2 to 3 ml urine is mixed with equal amount of 1 per cent barium chloride solution, the absence of any turbidity is an index of pregnancy. The method being simple does not require sophisticated equipments.

#### *Milk copper sulphate coagulation test:*

The test based upon the increase of gamma globulin in the milk of pregnant animals which reacts with copper sulphate and forms clots, while the milk of non pregnant animals remained as such.

Stancev and Angelov (1966) reported 90 to 92% accuracy at 30 to 40 days of pregnancy. Temblador and Acosta (1971) reported 86.36% reliability at 15 to 60 days of gestation. Abiley and Roussel (1975) recorded 50, 85, 67, 58 and 40% accuracy at 1 to 29, 30 to 45, 46 to 60, 61 to 90 and 91 and above days of pregnancy. In present study 52.88, 64.42 and 75.96% accuracy was recorded at 15 to 25, 26 to 35 and 36 to 45 days of pregnancy respectively.



The highest percentage of accuracy in the milk copper sulphate coagulation test was only 75.96% at 36 to 45 days of pregnancy with fairly high percentage of false negative reactions. The test is not much reliable for pregnancy diagnosis in cattle.

#### Acknowledgement

Thanks are due to Dr. Mohan Sinh, Dean, College of Veterinary and Animal Science, Bikaner and Dr. S.L. Bohra, Superintendent, Cattle Breeding Farm, Nagaur for their help and the facilities provided.

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## SHORT COMMUNICATIONS

### Epidermoid Cyst of the Ovary in a Gir Cow-A Clinical Report

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A Gir-cow-Vidula, which had last calved in May 1982, was offered for examination on 2.11.1982 as she had not exhibited oestrus even after 165 days of post-partum period. She was a regular calver and had seven calvings and was a moderate milker.

On rectal palpation, the left ovary could be felt to be enlarged and having cysts of varying sizes and consistency. The right ovary was inactive. The uterus was having a normal feel.

It was decided to remove the abnormal ovary expecting that normal reproduction will be established with the remaining ovary. Accordingly, the left ovary was removed on 20.11.1982 by resorting to laparotomy and the recovery of the animal was uneventful.

The gross description of the left ovary was as follows:

Length	5.0 cm.
Height	4.5 cm.
Breadth	3.5 cm.
Circumference	13.0 cm.
Weight of ovarian tissue	8.800 gm.
Weight of waxy material	27.550 gm.

Eight cysts and cystic cavities could be seen. One of the largest cyst was having the circumference of 11.0 cm. and con-

tained reddish brown waxy mass. The cyst walls were smooth.

A histological study of the left ovary showed the presence of luteal tissue and corpus albicans. Majority of the cysts seemed to be lined by flattened epithelium or granulosa cells arranged in one or two layers, a picture characteristic of follicular cysts. Some of the cysts were lined by stratified squamous epithelium; however there were no dermal appendages to categories such as cysts as dermoid cysts.

The tissue sections were sent to Royal Veterinary College, Sweden for confirmation and they reported it to be as "Epidermoid Ovarian Cysts".

The only record about the epidermoid cyst is by Hansen (1982), in buffaloes. He carried out patho-anatomical investigations of reproductive organs of Egyptian buffaloes and reported the incidence of epidermoid and dermoid cysts as 2.17%. There are reports regarding the dermoid cysts in buffaloes by Sane *et al* (1958) 1.2% and Sharma *et al* (1968) as 0.4% indicating the existence of dermoid cysts in buffaloes. There is no Indian record mentioning the existence of epidermoid cysts in either cows or buffaloes.



Hansen (1982) reported that dermoid and epidermoid cysts were found in the left ovary as often as in the right, some times in both ovaries and the size varied between that of a child's head down to a pea size. The consistency was doughy and the contents pasty or greasy. In the dermoid cysts hair could be found. He further stated that the presence of the cyst did not exclude the simultaneous presence of ripening follicles or luteal bodies or even pregnancy.

The present study of the cyst agrees with observations of Hansen (1982). The epidermoid cysts were found in the left ovary. The contents were semisolid, waxy, reddish brown in colour and odourless and the presence of luteal bodies could be detected.

Interestingly, similar epidermal inclusion cysts have been described in the dog while their origin is not fully explained. They are thought to arise from occlusions of hair follicles and subsequent isolation of the epithelium, so that the desquamating keratin accumulates in the cyst, causing it to enlarge.

This may be an acquired condition or may follow isolation of cell rests of abortive hair follicles during embryonal development and subsequent growth at a later stage (Mulligan, 1949; Smith *et al*, 1972).

The origin of the ovarian cyst described above is purely speculative.

The development of dermoid cysts of the ovary has been ascribed to partheno-

genetic spontaneous development of an unfertilized totipotential ovum (Robbins, 1960; Boyd, 1961).

After the removal of the left ovary, the right ovary has become activated and the animal has exhibited first oestrus after operation on 10th December '82 and subsequently she exhibited oestrus on 10th January and 1st Feb. 1983, She was inseminated on 10th Jan. and on 1st Feb. She has remained pregnant to the last insemination. This implies that the earlier anoestrous condition was most probably due to hormonal imbalance caused by the abnormality of the left ovary which might have caused the right ovary to remain non-functional.

Such cysts are understandably devoid of any endocrine activity and therefore the anoestrous condition of the animal could be ascribed only to the hormonal effects of follicular cysts. This argument is sustained by the observation that the animal had already calved seven times before and further it again came into heat after removal of the affected ovary.

The above findings indicate the possibility of the existence of other types of ovarian cysts. As such it is necessary to ascertain the type of the cyst before remedial measures are resorted to.

### Acknowledgement

Thanks are due to Dr. B.V. Jalnapurkar, Prof. of Pathology, for his keen interest and active co-operation.

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## The Uterine Rupture in a Cow-A Case Report

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Rupture of the gravid uterus can occur due to extreme violence and trauma in advanced pregnancy. In rare instances foetal emphysema may cause rupture of the uterus (Roberts 1956). Incidence of rupture of uterus due to foetal emphysema consequent to trauma being rare in the practice such case is recorded here for the benefit of the bovine practioners.

A Sindhi cow belonging to the regional Research Station of UAS Dharwar was presented with history of anorexia and tympany. The clinical examination revealed that there was acute tympany with hypermotility of the rumen. The body temp. was normal (101.8°F), pulse and respirations was rapid and shallow respectively. The frank gas in the rumen caused respiratory distress. Therefore gases were removed immediately by needle puncture of the rumen and oil turpentine 30 ml was injected intraruminally. Oral antizymotics were administered. The relief of tympany was short lived and again after 3 hours it showed the same signs. At this stage, rectal examination showed the rumen occupying the space even in the pelvic cavity with gases. The body of the uterus enlarged but doughy in nature and no frimitus could be felt. History revealed that the cow was pregnant by 5-6 months, and had a severe fall and both hind limbs were abducted 24 hours before the appearance of the tympany. Vaginal examination showed absence of cervical seal but there was no relaxation of cervical

walls. But after 24 hours after 1st examination, the cow was found to be recumbent and the cow appeared to be in agony with difficulty in breathing and rapid pulse rate. The tympany continued to



Ruptured portion of uterus and foetus in the abdomincavity with intact umbilical cord

remain without any ruminal movements. The vaginal examination revealed slight relaxation of cervix. The cow was given fluids parenterally and antibiotics were administered to combat infections. Vetoestrol 90 mgm I/m was administered followed by carbochol 1.5 ml 6 hours after S/cut.

After 44 hours, the fluid therapy was repeated and cervical relaxation was evident and fist could be passed without difficulty, but no fetal part could be palpated in the uterus. The foul smell emanated from the uterus and cotyledon came with the hand showed signs of



putrefaction and completely discoloured. This was suggestive of a rupture of the uterus.

As the condition of the cow was fast deteriorating with signs of toxinaemia which prevented any surgery because of poor risk. Therefore fluids and antibiotics were repeated.

After 72 hours after the treatment, the cow showed no response but condition continue to deteriorate and the cow died by 80 hours.

The post-mortem examination was conducted and following observations were made.

The female foetus with umbelical cord

intact was found free in the abdominal cavity (Figure-1). The tunica was full of sero-Sanguinous fluid with offensive odour. The foetus showed emphysematous condition with advanced degree of putrefication. The uterus showed rupture.

From the progress of the case and history of accidental injury it seems that foetus died due to severe trauma and subsequent emphysema resulted into the tear of the uterus resulted into general peritonities or toximia and death.

It may be concluded that an early laparotomy might have saved the cow. Tympany however simple it might appear in a pregnant animal needs to be carefully evaluated.

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## Influence of Bulls and the Periods of Inseminations on Fertility in Surti Buffaloes

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Perusal of the scientific literature did not reveal much information on influence of bulls and the periods of inseminations on fertility in buffaloes under field conditions (Hafez, 1953; Sambashiv Rao, 1970; Gill, 1973; Abhi *et al.*, 1973).

### Materials and Methods

The data available on 44 bulls and for six periods were utilised. A study was undertaken on the following information:

The number of conceptions between bulls or periods could not be compared as such since the number of inseminations varied for different bulls. Hence the 'F' test using 'Analysis of Covariance' was employed (Zar, 1974). This method compared the number of conceptions after suitably adjusting for the corresponding number of inseminations. After adjusting for the number of inseminations, the number of conceptions per bull was esti-

Item	P E R I O D S (in hours)								
i. intervals in hours	0-6	6-12	12-18	18-24	24-30	30-36	36-42	42-48	Total
ii. Insemination fresh	6	15	101	264	110	4	0	0	500
iii. Conceptions	1	5	51	160	57	1	0	0	275
Per cent	16.66	33.33	50.49	60.60	51.81	25	0	0	AV:55

(Madhu Rao, 1976)

a. ANOVA					X—Number of inseminations Y—Number of conceptions		
SOURCE	DF	SS(X)	SP(XY)	SS(Y)			
Bulls	43	446.0304	240.6667	167.7083			
Periods	5	1150.1667	700.6667	428.9280			
Error	215	956.8333	528.8334	405.9054			
Total	283	2553.0304	1470.1667	1002.5417			

b. ANOVA						
SOURCE	DF	SS(Y)	MS	F	DF	P
Bulls (c)	43	37.9030	0.8815	1.660	43;214	<0.05
Periods (c)	5	3.7581	0.7516	1.416	5;214	(0.2—0.5)
Error (c)	214	113.6238	0.531			

Under Hypothesis			F value	Degrees of freedom	P value
a.	No difference between the bulls on the number of conceptions effected		1.660	43;214	<0.05
b.	No difference between the periods on the number of conceptions		1.416	5;214	0.2—0.5



Sr. No.	Bull number	Number of conceptions (adjusted)	Sr. No.	Bull number	Number of conceptions (adjusted)
1.	3	9.9	23.	11	6.5
2.	39	9.8	24.	7	6.4
3.	16	9.7	25.	14	6.2
4.	4	9.5	26.	21	6.2
5.	34	9.2	27.	22	6.1
6.	18	8.6	28.	1	5.9
7.	44	8.4	29.	19	5.8
8.	42	8.2	30.	6	5.6
9.	25	8.1	31.	40	5.6
10.	36	7.8	32.	46	5.5
11.	17	7.6	33.	31	5.5
12.	8	7.5	34.	29	5.2
13.	2	7.4	35.	20	4.9
14.	23	7.1	36.	30	4.8
15.	41	7.1	37.	38	4.4
16.	45	7.0	38.	26	4.3
17.	28	6.9	39.	10	4.0
18.	33	6.8	40.	43	4.0
19.	36	6.7	41.	27	3.1
20.	13	6.7	42.	12	2.5
21.	32	6.6	43.	24	1.8
22.	35	6.6	44.	15	0.0

mated from the formula:

$$Y_i(e) = Y_i - b \left( X_i - \frac{X_{..}}{44} \right)$$

where  $Y_i(e)$  = Adjusted value of number of conceptions for  $i$ th bull

$Y_i$  = Observed value of number of conceptions for  $i$ th bull

$b$  = Regression coefficient

$X_i$  = Number of inseminations for  $i$ th bull

$X_{..}$  = Total number of inseminations for all the 44 bulls

Substituting for  $b$  &  $X_{..}$ , the above equation becomes:

$$Y_i(e) = Y_i - 0.5527X_i + 6.2807$$

The data was processed through IBM-1620 Computer by standard statistical methods (Snedecor and Cochran, 1967).

## Results and Discussion

Analysis details:

### Conclusion

a. The difference between the BULLS IS SIGNIFICANT

b. The difference between the PERIODS IS NOT SIGNIFICANT

In the following table, the bulls have been ranked according to the adjusted number of conceptions effected:

From the above results it is evident that most of the farmers are bringing their buffaloes for inseminations in all probabilities at the opportune time. It can also be seen from the table in the materials that the number of inseminations carried out in the very early or very late part of the oestrus are limited. It is further observed that there is a great variation in the fertility ability between



the bulls. The variation is too wide which is evident from the fore-going table. This points out the possibility for usage of the high fertile bulls on a large scale and the removal of the economically infertile bulls.

### Summary

Under the conditions prevailing where the study was undertaken on a data of 500 fresh artificial inseminations with 44 bulls, the difference in fertility between the bulls was significant where as the

difference between the periods was not significant. This points out to the rearing intensities practised in buffaloes and the need to use high fertile bulls and removal of the economically low fertile bulls.

### Acknowledgement

Thanks are due to the authorities of the Gujarat Agricultural University for the provision of the facilities for the studies. Thanks are also due to the Staff, Dept. of Gynaecology & Obstetrics Veterinary College, Anand for all the help.

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## Conception and Calf Born Percentage in Cattle and Buffaloes for Artificial Insemination with frozen semen :

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The advent of Artificial Insemination by itself is a boon to dairy farmers and the latest introduction of Frozen Semen technology in the field of Artificial Insemination has further exalted the advantages particularly in the developing and under developed countries. This technology is bound to create the much sought white revolution in our country in the near future; with the present emphasis on productivity, artificial insemination with deep frozen semen is sure to bring in fruitful results.

The birth of first calf due to artificial insemination with frozen semen in cattle was reported from England by Stewart (1951). The addition of 10 per cent glycerol to the semen prefreeze has improved the conception rate (Graham and Marion, 1953). As reported by Kumaran (1965) and Bhosrekar (1973), the results with Deep Frozen Semen have not been encouraging. Qureshi (1979) has reported a higher conception rate with deep frozen semen of Brown-Swiss bulls. Dutta *et al* (1980) reported that the months and seasons exercise an effect on the conception rate. There was a significant effect of age of cattle on the conception rate (Dutta *et al* loc. cit.). Sharma *et al* (1979) observed that the conception rate with Egg Yolk Citrate (frozen) was higher than that of Citric Acid Whey (frozen) in buffaloes.

### Materials and Methods

The Frozen Semen Bank, Hebbal, Bangalore, has been deep freezing semen from bulls and buffaloes successfully since 1977. The method of freezing of semen is as per Landhsut method.

This Institution has been supplying frozen semen and Liquid Nitrogen to 80 Artificial Insemination Centres operating in I.C.D.P. area of Bangalore and whose results are being monitored at this Centre. The data from these 80 Centres forms the basis for the present study.

The conception and the calf born rate are calculated as follows:

$$(1) \% \text{ Conception} = \frac{\text{No. of animals conceived}}{\text{No. of animals inseminated \& examined}} \times 100$$

$$(2) \% \text{ Calf born} = \frac{\text{No. of calves born}}{\text{No. of animals inseminated \& examined}} \times 100$$

Table shows the A.I. done and fertility results in Cattle and buffaloes due to A.I. with deep frozen semen from 1977-78 to 1981-82.

It is evident from the table, that the conception rates in cattle varied from 43.39 per cent to 62.84 per cent with a overall mean of 56.33 per cent. Simi-



TABLE SHOWING THE NUMBER OF ARTIFICIAL INSEMINATION DONE AND FERTILITY RATE IN CATTLE AND BUFFALOES

	CATTLE						BUFFALOES					
	1977-78	1978-79	1979-80	1980-81	1981-82	TOTAL	1977-78	1978-79	1979-80	1980-81	1981-82	TOTAL
1. No. of Centres using Frozen Semen.	54	68	80	80	80	—	54	68	80	80	80	—
2. No. of cases Inseminated.	67430	70671	103465	125077	111575	478218	10654	9078	17909	24033	13633	75307
3. No. of cases followed up.	13122	34606	61156	73699	63468	246051	2074	4446	8153	11093	9527	35292
4. No. of cases found pregnant.	6927	15014	33782	43014	39883	138620	830	1778	4283	5651	4614	17156
5. Percentage of conception	52.79	43.39	55.24	58.36	62.84	56.33	40.02	40.00	52.53	50.95	48.43	48.61
6. No. of calves born.	4724	12458	22109	28553	32336	100180	746	1600	2944	4224	4154	13668
7. Percentage of calf born.	36.00	36.00	36.15	38.74	50.94	40.71	35.97	35.99	36.11	38.08	43.60	38.72



larly the calf born percentage varied from 36.00 per cent to 50.94 per cent in cattle with an overall mean of 40.71 per cent.

The conception rate in buffaloes varied from 40.00 per cent to 52.53 per cent with an overall mean of 48.61 per cent and the calf born percentage varied from 35.97 per cent to 43.60 per cent with an overall mean of 38.72 per cent.

### **Summary**

Conception rate in A.I. with deep

frozen semen in cattle and buffaloes is reported and the results have been encouraging.

### **Acknowledgement**

The authors express their deep sense of gratitude to Dr. G.M. Srikantaiah, Director, Animal Husbandry and Veterinary Services in Karnataka, Bangalore, for having accorded permission to publish the article.

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## Effect of Experimental *T. Evansi* Infection on the Reproductive Organs of Albino Mice, Rats and Rabbits, A Preliminary Note.

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Studies on pathology of reproductive organs in trypanosomiasis have been reported by Isoun and Anosa (1974) in sheep and goats infected with *T. vivax*. A similar study was undertaken by Losos and Ikede (1970) in sheep and goats, Ikede (1979) in sheep, Moulton and Sollod (1976) in calves, Ingh and Dijk (1975) in rabbits infected with *T. brucei*. The present communication deals with such study in experimental trypanosomiasis in albino mice, rats and rabbits.

Fifteen albino mice were inoculated intraperitoneally each with 2.0 ml of blood of infected buffalo calves with *T. evansi*. (Wet blood film examination of buffalo calves however did not reveal *T. evansi*). 0.5 ml of infected mice blood containing  $1.7 \times 10^4$  trypanosomes was inoculated intraperitoneally into 15 albino rats and 6 rabbits. Two mice and one rat were euthanized at 48 hours interval while one rabbit at 15 days interval. Control animals one from each species were also euthanised simultaneously. The gross lesions were noted. Tissues from testes, ovary, Fallopian tube and uterus were collected in 10% formalin, were processed by routine paraffin embedding method and stained with Harri's haematoxyline and eosin method.

No gross lesions were noticed during the initial stage of disease. Congestion of

the superficial blood vessels of testes was noticed after 9th and 17th day post inoculation (P.I.) in mice and rats respectively. In rabbits swelling of the scrotum was noticed on 32nd day P.I. which later on turned in to exudative dermatitis. In female swelling of vulva was observed after 38th day P.I. and severe inflammation of vulva was observed in the rabbit on 84th day P.I. Similar lesions have been described by Ingh and Dijk (1975) in rabbits infected with *T. brucei*. Isoun and Anosa (1974) reported testicular atrophy, scrotal alopecia, calcification and occasionally infarction of testicle in sheep and goats infected with *T. vivax*.

Microscopically trypanosomes were found in the blood vessels on 7th and 11th day P.I. in testes of mice and rats respectively. On 11th and 13th day P.I. in mice and rats respectively, they were also found in haemorrhagic areas. Losos and Ikede (1970) in sheep and goats and Ikede (1979) in sheep infected with *T. brucei* also observed similar lesions in testes. Lesions noticed in testes were testicular degeneration, focal haemorrhages, occasionally oedema in the testicular stroma associated with infiltration of mononuclear cells and eosinophils and absence of spermatozoa or spermatid. Similar lesions were noticed by Moulton and Sollod (1976) in calves infected with

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*T. brucei*. On the 11th day P.I. in mice and 19th day P.I. in rats, necrosis of seminiferous tubules was evident. Such lesions were also reported in *T. brucei* infection of rabbit (Ingh, 1977), rams (Ikede, 1979) and *T. congolense* infection of goats (Kayya and Odour-Okede, 1980).

In female, trypanosomes were found to be present in the blood vessels and in the haemorrhagic areas with degenerative changes in the ovary, Fallopian tube and uterus. Desquamation of superficial epithelium and glandular epithelium was observed in uterus by the end of 9th, 15th and 45th day P.I. in mice, rats and rabbits respectively. Infiltration of mononuclear cells, eosinophils and occasional plasma cells was observed in ovary, Fallopian tube and uterus with congestion and focal haemorrhages. Molton and Sollod (1976) noticed mononuclear and eosinophilic infiltration with oedema in the uterus of calves infected with *T. brucei*.

It can be emphatically said that there are chances of transmission of trypanosomes to the embryo as trypanosomes were found in the reproductive organs. Vrijburg (1900) infected a mare by rubbing urethral secretion of a surra affected stallion into vaginal mucosa. According to Sergeant *et al.* (1919) during the acute stage of infection, trypanosomes were transported via placenta to foetus in camels. The possibility of transplacental transmission of surra in dogs and guinea-pigs was established by Kraneveled and Mansjoer (1954) by experimentally infecting pregnant bitches and guinea-pigs with *T. evansi*. However, similar experiments in rabbits and rats proved unsuccessful (Gill, 1977). Betancourt (1978) observed severe *T. T. vivax* parasitemia in a day old calf during an outbreak of trypanosomiasis and considered that the parasites must have been transmitted through uterus. Hence further study on transplacental transmission of *T. evansi* is suggested.

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## Reproductive Performance of Infertile Cattle Induced to Lactation

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### ABSTRACT

Thirty five infertile cattle were induced to lactation to evaluate the effect of induced lactation on reproduction. Twenty one animals were allotted to experimental group and 14 to the control group. Animals were divided into groups namely: Gr. 1-Cystic Ovary, Gr. 2-Persistent Corpus Luteum, Gr. 3-Subactive/Inactive Ovaries and Gr. 4-Repeatbreeder. Lactation was induced in experimental animals by administration of diethylstilboestrol (@ 7 mg/100 kg body wt.) and progesterone (@ 20 mg/100 kg body wt.) for 12 days followed by prednisolone (@ 20 mg/animal/day) on day 13 to 15 and reserpine (@ 3 mg/animal/day) on day 17 to 19.

Fourteen out of 21 animals (66.6 per cent) in experimental group conceived within 45 days after the last reserpine injection. In control group only one animal (7.1 per cent) conceived till 120th day of post-therapy. There was no physical injury in any animal due to oestral activities. The hormonal regime reported for induction of lactation could be of special importance in handling the infertility problems in cattle of high genetic value.

\* \* \*

High doses of oestradiol and progesterone have been used by numerous investigators to induce lactation in non-preg-

nant, non-lactating dairy cows (Erb *et al.*, 1973; Smith and Schanbacher, 1973; Collier *et al.*, 1975, 1977; Chakriyat *et al.*, 1978). Exogenous hormones alter the indigenous hormonal balance favouring the growth and secretory activities in mammary glands. These also interact with functioning of ovaries, pituitary and possibly other endocrine glands with variable consequences. Field *et al.*, (1980) did not find any effect of induced lactation on reproduction. Collier *et al.*, (1975) using 7 day treatment schedule of induced lactation observed that the treatment resulted into regression of ovaries in all animals with 1st oestrus occurring on an average of 43 days following the last oestrogen-progesterone injection. They bred 9 cows of which 5 conceived with no complications during pregnancy. Harness *et al.*, (1978) induced lactation in five cyclic heifers of which four had no effect on ovarian cyclic activity while the fifth developed cystic condition. In his experiment another heifer with cystic ovarian condition became static.

Maurya (1980) reported conception in one of the twelve infertile cows induced to lactation with oestrogen-progesterone and prednisolone given over a period of 13 days.

### Materials and Methods

Thirty five infertile cattle free from



TABLE 1. CATTLE INDUCED INTO LACTATION

Sr. No.	Group	No. of animals			Control		
		Experimental Heifer	Cow	Total	Heifer	Cow	Total
1.	Gr. 1: Cystic Ovary	1	1	2	Nil	2	2
2.	Gr. 2: Persistent Corpus Luteum (PCL)	3	2	5	2	2	4
3.	Gr. 3: Subactive/Inactive Ovaries	6	Nil	6	4	Nil	4
4.	Gr. 4: Repeat Breeder	1	7	8	1	3	4
Total:		11	10	21	7	7	14

permanent anatomical deformities were included in the present study. Animals were between 3 to 7 years of age and were kept in open barns at Livestock Research Centre (L.R.C.) of the University under the standard managerial conditions. Animals were divided into four groups on the basis of changes in their reproductive organs which were decided following three clinical examinations held at an interval of 3 days along with the previous reproductive history. Animals in each group were allotted on at-random basis to either the experimental or control group (table 1). All animals had been infertile for atleast 6 months before being included in this study.

Experimental animals were subjected to artificial induction of lactation by administration of a combination of diethylstilboestrol (Vetosterol, May & Baker) and progesterone (Proluton depot, German Remedies) @ 7 mg/100 kg body wt. and 20 mg/100 kg body wt., respectively for 12 days, followed by prednisolone (Hostacortion 'H', Hoechst) @ 20 mg/animal/day on day 13 to 15 and reserpine (Reserpine, Inverni & Della Beffa Sp A, Italy) @ 3 mg/animal/day on day 17 to 19. Post-therapy reproductive status including ovarian changes were ascertained by weekly clinical

examinations. Animals were examined two times every day for signs of oestrus and were bred by artificial insemination if detected in oestrus. Animals with established pregnancy were clinically examined at 15 day interval and on day 100 and day 120 post-therapy.

Animals were hand milked. Data on milk production would be reported separately.

### Results and Discussions

All experimental animals exhibited variable degrees of behavioural signs of oestrus between 2nd and 5th day of the hormonal therapy except the animals of cystic ovarian group in which these signs were intense and extended upto 9th day post therapy. There was no physical injury in any animal due to oestral activities.

Fourteen of the 21 animals induced lactation (66.6 per cent, 9 heifers and 5 cows) conceived within 45 days after the last reserpine injection (Table 2). At day 100 post-therapy, pregnancy was recorded in none out of 2 animals (0 per cent), 4 out of 5 animals (80.0 per cent), 5 out of 6 animals (83.5 per cent) and 5 out of 8 animals (62.5 per cent) in group with the history of cystic ovaries, persist-



TABLE 2. REPRODUCTIVE PERFORMANCE OF CATTLE  
INDUCED TO LACTATION

Sr. No.	Animal No.	Cow/heifer	Post-therapy reproductive status at day 100	ovarian status at day 120
Gr. 1: Cystic Ovaries:				
1.	1073	Heifer	N.P./subactive	N.P./sub active
2.	560	Cow	N.P./subactive	N.P./cystic
Gr. 2: Persistant Corpus Luteum (PCL):				
1.	650	Heifer	Pregnant	Pregnant
2.	715	Cow	Pregnant	Pregnant
3.	866	Cow	N.P./Cyclic	N.P./Cyclic
4.	1326	Heifer	Pregnant	Pregnant
5.	1537	Heifer	Pregnant	Pregnant
Gr. 3: Sub-active/inactive ovaries:				
1.	649	Heifer	N.P./no change	N.P./no change
2.	E922C/1160	Heifer	Pregnant	Pregnant
3.	E945 B/	Heifer	Pregnant	Pregnant
4.	1324	Heifer	Pregnant	Pregnant
5.	1525	Heifer	Pregnant	Pregnant
6.	1728	Heifer	Pregnant	Pregnant
Gr. 4: Repeat breeder:				
1.	246	Cow	N.P./no change	N.P./no change
2.	267	Cow	Pregnant	Pregnant
3.	511	Cow	Pregnant	Pregnant*
4.	737	Cow	N.P./no change	N.P./no change
5.	756	Cow	Pregnant	Pregnant**
6.	959	Cow	N.P./no change	N.P./no change
7.	977	Cow	Pregnant	N.P./PCL***
8.	1114	Heifer	Pregnant	Pregnant

N.P. = Non pregnant \* aborted at 7½ months of gestation

\*\* gave birth to female twins. \*\*\* embryonic death.

ant corpus luteum, subactive/inactive ovaries and repeat breeding, respectively. One of the pregnant cows aborted at 7½ months and another had embryonic death during 4th month of gestation. One cow gave birth to female twins.

In animals with history of cystic ovarian condition, the follicular cysts regressed within 15 days post-therapy resulting into small and smooth ovaries. At day 120 post-therapy ovaries remained subactive in one animal while in the other there

was reoccurrence of follicular cyst. In the nonpregnant cow with the history of persistant corpus luteum, ovaries became cyclic although the animal did not conceive. In cow No. 977 with history of repeat breeding the embryonic death was recorded to have occurred between day 100 and 120 post-therapy. In the remaining animals which did not conceive, there was no change in the ovarian activity.

In control animals only one animal



(heifer, repeat breeder) conceived. In the remaining nonpregnant animals there was no change in the ovarian activity.

Lembowicz *et al.* (1982) recorded pregnancy in 7 out of 10 infertile cows synchronized with PG F<sub>2</sub> analogue and induced to lactate with a short oestradiol and progesterone therapy (less than 5.5 days) followed by a long reserpine treatment (atleast 7 days). In other groups of animals induced with long (7 days) oestradiol -progesterone regime, seven of the 8 cows developed cystic ovarian condition and none conceived. However, the basic causes of infertility in experimental cows had not been recorded by these workers. In the present experiment an

improved fertility rate and absence of cystic ovarian condition during the post therapy period has been recorded in animals with previous history of PCL, subactive/inactive ovaries and repeat breeding which can be attributed to low daily amount of oestrogen injected and is in agreement with findings of Lembowicz *et al.*, (1982).

The data presented here indicate that 19 day regime of low doses of steroids and reserpine used for artificial induction of lactation has obvious advantage that in addition to milk production it improves the fertility level in infertile animals and thus has special importance in handling the infertility problems in cows of high genetic value.

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## "Studies on Biometry of Testis and its Correlation to Sperm Producing Ability in Surti Buffalo Bulls."

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### ABSTRACT

A study on biometry of testis, predicted testicular weight and their correlation to sperm producing ability in 32 Surti buffalo bulls was undertaken. The mean predicted testicular weight was  $213.6637 \pm 9.2689$  gms. Average weight of left testis was higher (113.86 gms) than the right testis (99.848 gms.). The mean transverse scrotal circumference was 36.281 cms.

Statistical analysis revealed correlations between body weight and testicular weight ( $0.983 \pm 0.035$ ), transverse scrotal circumference and testicular weight ( $0.997 \pm 0.015$ ); and body weight and transverse scrotal circumference ( $0.986 \pm 0.031$ ). Testicular weight and scrotal circumference also revealed highly positive correlation ( $0.962 \pm 0.052$ ) with the sperm concentration in semen.

\* \* \*

Information on the biometry of testis and their correlation to sperm producing ability in buffalo bulls is scanty, (Yassen & Mahmoud 1972; Ansari et.al. 1972). An effort was made to study these correlations in surti breed buffalo bulls.

### Materials and Methods

A total of 32 Surti buffalo bulls were studied for drawing correlations of predicted testicular weight and transverse

scrotal circumference with sperm concentration in the semen. The age of these bulls studied ranged from 26.00 to 131.00 months. These bulls were thoroughly screened for general and special sexual health and were found healthy. To calculate body weight of the bulls; length and girth were measured with tape measure and calculation were done using Shaeffer's formula as cited by Sastry and Thomas (1976), although the formula is meant for cattle. Testicular biometry was done according to Rosenberger (1979) and calculated weights were predicted using regression equation (Ansari et.al. 1972):

$$\bar{Y} = -254259 + 1.060435 \cdot \log X_1 + 0.358399 \cdot \log X_2 + 1.180849 \cdot \log X_3$$

where:

$\bar{Y}$  = predicted testicular weight,

$X_1$  = length of the testis,

$X_2$  = Width of the testis,

$X_3$  = thickness (depth of the testis).

Sperm concentration was estimated using improved Neuber chamber haemocytometer. Eosin Y0.25 gm (water soluble) in 100 ml. of formal saline (1 ml. formalin + 99 ml. of normal saline) was used for the purpose, (Tomar 1970).

All the statistical analysis was carried out according to Snedecor and Cochran (1971).



## Results and Discussion

The calculated mean body weight was  $388.703 \pm 69.83$  Kg and these observations ranged from 256.227 to 514.510 Kgs. The means and range of length, width, depth and predicted weight of right testicles were 12.219 cms, 10.00 to 16.00 cms; 6.594 cms, 5.00 to 12.00 cms; 4.828 cms, 4.00 to 6.00 cms and 99.848 gms, 57.997 to 160.00 gms. Whereas the means and range for left testicles were 13.703 cms, 10.00 to 17.00 cms; 6.078 cms, 5.00 to 9.00 cms; 5.141 cms, 4.00 to 6.50 cms and 113.816 gms 72.327 to 169.57 gms.

The calculated mean testicular weight was  $213.664 \pm 9.269$  gms. The present findings compare well with the findings of Joshi et.al. (1967) and Maurya et.al. (1968). However the readings in the present study are on live bulls while the readings compared are from post-mortem study.

Statistical analysis for correlation between body weight and total testicular weight revealed a highly positive correlation. The coefficient of correlation was  $0.983 \pm 0.035$ . Transverse scrotal circumference and testicular weight revealed highly significant correlation. The coefficient of correlation was  $0.997 \pm 0.015$ . A highly significant positive correlation,  $0.986 \pm 0.031$  was found between body weight and transverse scrotal circumference.

Yassen and Mahmoud (1972) and Ansari et.al. (1972) have drawn correlations between body weight and testicular size in buffalo bulls. The observations in

the present study compare very well with their findings. Also in the present study a highly significant positive correlation was observed between scrotal circumference and testicular weight and body weight and scrotal circumference. Similar observations were made by Yassen and Mahmoud (1972) and Ansari et.al. (1972).

Statistical analysis revealed a highly significant, positive correlation between total testicular weight and sperm concentration. The coefficient of correlation worked out to be  $0.962 \pm 0.052$ . This finding is in agreement with the trend reported by Ansari et.al. (1972).

The correlation between transverse scrotal circumference and sperm concentration was  $0.962 \pm 0.052$ . Yassen and Mahmoud (1972) and Ansari et.al. (1972) also found a similar trend and the present findings are in close agreement with them.

The results of the present study under report indicated that transverse scrotal circumference measured the sperm producing ability in the Surti buffalo bulls. With a normal health, transverse scrotal circumference (TSC) can be used as an important criteria for knowing their sperm producing potentialities (SPP).

Thanks are due to Director, Animal husbandry, Gujarat state, Principal Gujarat Veterinary College, Anand, authorities of various semen banks for the help rendered and providing facilities for the study.

Thanks are also due to Dr. M.M. Patel and Dr. S.K. Dalal for help in analysing the data.



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# Chemical Constituents of Normal and Frozen Buffalo Semen

M.R. BHOSREKAR

National Dairy Research Institute, Karnal

Buffalo semen diluted in citric acid whey with glycerol (CAWG) (Ganguli *et al* 1973; Bhosrekar and Ganguli, 1976) was frozen by French Technique over liquid nitrogen vapour. The materials were as per Bhosrekar; (1980). The buffalo semen was diluted in the ratio of 1:10 and samples of semen were taken just before freezing (i.e. after processing the semen through glycerolisation and equilibration) and 24 hours after freezing and thawing. The extracellular fluid obtained after centrifugation for 30 minutes at 10,000 RPM in refrigerated centrifuge was used for estimations. Calcium, inorganic Phosphorus, magnesium were estimated by methods described by Hawk *et al* (1954). Sodium and Potassium was determined by flame photometer by using respective filters. Nitrogen and non protein nitrogen was estimated by micro kjeldhal method and citric acid and ascorbic acid was estimated by method described according to IDF 34 A (1967) and Gyorgy (19650) respectively.

After freezing of semen the extracellu-

lar fluid showed increase in sodium and potassium contents, while there were no differences in calcium, inorganic phosphorous and magnesium contents (Table I).

Graham and pace (1967) have shown slight release of potassium from cells after freezing and thawing. They did not record any change in calcium and inorganic phosphorous contents before and after freezing. Chaplin *et al* (1958) also had shown leakage of potassium from cells on freezing because of damage to cell wall. Similar finding was on record by love-lock (1967).

The amount of total nitrogen and non protein nitrogen was not found to differ after freezing similarly there was no significant difference in citric acid and ascorbic acid contents of extracellular fluid before and after freezing. However, there was decreasing trend seen as regards citric acid and ascorbic acid contents on freezing semen.

The author wishes to thank Dr. N.C. Ganguli for his guidance in this work.

TABLE 1. INORGANIC AND ORGANIC CONSTITUENTS IN BUFFALO SEMEN BEFORE AND AFTER FREEZING

Treatments	Potassium	Sodium	Mg per 100 ml			Total Nitrogen	Non-protein Nitrogen	Citric Acid	Ascorbic
			Inorganic	Calcium	Magne-sium				
Before	73.00	246.46	24.67	99.66	7.83	973.10	66.10	635.00	0.350 9
Freezing	± 18.50	± 35.80	± 1.73	± 6.20	± 0.29	± 161.30	+ 24.60	± 78.40	± 0.042 7
After	82.30	263.46	23.24	99.66	8.04	1001.70	67.00	598.88	0.330 8
freezing	± 12'00	± 45.90	± 1.76	± 5.20	± 1.04	± 143.10	+ 13.90	± 65.80	± 0.031 0
't' value	2.4009*	2.1963*				1.6485 NS	0.00 NS		
	n = 15	n = 15				n = 14	n = 14		

\*: Significant at 5 per cent level

NS: Non Significant.



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## Effect of Glycerolisation Procedures on Post Thaw Motility of Buffalo Spermatozoa

N. BHANDARI, R.A.S. CHAUHAN and ABRAHAM MATHEW

College of Veterinary Science and Animal Husbandry, Mhow (M.P.)

Glycerol plays an important role in the successful deep freezing of semen of different species of animals.

There are few reports on the techniques of glycerolization *vis-a-vis* post thaw motility of buffalo spermatozoa.

In the present investigation the effect of various ways of glycerolization on the post thaw motility of buffalo spermatozoa has been studied.

### Materials and Methods

Five Murrah buffalo bulls belonging to Indo-Swiss project Mattupatty were

included in the study. The semen was collected in the artificial vagina and from each bull six collections were taken.

The semen was diluted in Tris egg yolk dilutor to which glycerol was added in the following ways:

(i) The dilutor, Tris egg yolk was divided into two parts, A and B. The part A contained 6 per cent glycerol and part B 6.8 per cent glycerol. The part B was mixed with part A in two steps in two equal volumes after the dilution of semen in part A, at an interval of 10 minutes.

TABLE 1. PRE-FREEZING AND POST THAW MOTILITY AFTER GLYCEROLISATION.

Sr. No.	Method of glycerolisation (treatment)	Pre-freezing motility (per cent $\pm$ SE)	Post thaw motility (per cent $\pm$ SE)
1.	6% glycerol in part A of diluter and 6.8% in part B.	68.91 $\pm$ 3.152	37.40 $\pm$ 3.873
2.	12.8% glycerol in part B diluter, glycerolisation in single step.	66.85 $\pm$ 2.045	32.228 $\pm$ 4.634
3.	12.8% glycerol in part B diluter glycerolisation in two steps.	67.80 $\pm$ 2.436	31.998 $\pm$ 3.23
4.	12.8% glycerol in part B diluter, glycerolisation in four steps.	68.80 $\pm$ 2.028	36.50 $\pm$ 2.90

TABLE 2. ANALYSIS OF VARIANCE

S.No.	Source of variation	d.f.	MS	F value
1.	Between bulls	4	366.5742**	8.306519
2.	Between collections	5	489.5187**	11.092425
3.	Between treatments	107	44.1309	

\*\* P < 0.01



(ii) Part A of the dilutor was without glycerol whereas part B contained 12.8 per cent glycerol. After the initial dilution of semen in part A the part B was added to part A in a single step.

(iii) The part A and B of the dilutor were as in (ii) above but the part B of the dilutor was added to part A in two steps, each of equal volume at an interval of 10 minutes.

(iv) The composition of part A and B of the dilutor was as in (ii) above, but part B of the dilutor was added to part A in four steps, each of equal volume at an interval of 10 minutes.

The glycerolated semen was equilibrated for hours and frozen in liquid nitrogen using medium french straws. The post thaw motility was observed under phase contrast microscope.

## Results and Discussion

Table-1 presents the pre-freezing and post thaw motility of the spermatozoa

in various treatments. It is seen from this table that in treatment 1, maximum pre-freezing ( $68.91 \pm 3.152$  per cent) and post thaw motility ( $37.40 \pm 3.873$ ) was observed.

Analysis of variance (Table-2) revealed that there was no significant differences between treatments, though highly significant ( $P < 0.01$ ) difference was observed between bulls and between collections.

Our results are in agreement with the findings of Vasanth (1979), Flukiger *et al.* (1976) and Singh Sall *et al.* (1980), though Bandopadhyaya (1975) and Pandit *et al.* (1977) reported slightly higher levels of glycerol for effective deep freezing of buffalo semen. Md. Shafi Ch. (1979) found that 5 per cent glycerol is suitable.

The results of the present study do not support the findings of Sahani and Roy (1972) as they could not get satisfactory results by glycerolating the semen in various steps.

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## **Blood Serum Profile in Calves and Postpartum Buffaloes (Surti breed) with Associated Peridata Related to Reproductive Efficiency**

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*Major Advisor* Dr. K. Janakiraman

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The study involved calves from birth to maturity and postpartum buffaloes. The experiment on calves indicated the potentiality of Surti buffalo calves—both sexes—to attain puberty and sexual maturity between 14 to 17 months of age, with sufficient body weight and growth of reproductive organs. In such calves 13 biochemical characteristics were studied from birth to maturity. The serum alkaline phosphatase (AKP), protein bound iodine (PBI), calcium and inorganic phosphorus showed decrease towards puberty and sexual maturity. Total cholesterol, ester cholesterol and total protein increased towards puberty and maturity. An increasing trend around maturity was shown by serum copper and iron. Serum chloride tended to decrease towards puberal age. Magnesium in circulation tended to maintain at constant level. Significant sex difference in the serum levels of total protein, calcium and magnesium was recorded. Serum testosterone peaks were found to be important for the attainment of puberty.

The need for the early feeding of colostrum after birth for the essential biochemical adjustments for the survival and better growth was stressed.

The experiment on postpartum buffaloes was conducted to know the biochemical

and hormonal profile from calving to 1st postpartum heat and uterine involution. Apart from the biochemical characteristics studied in calves, the serum oestradiol—17B and progesterone were assayed to monitor postpartum events relevant to reproductive efficiency. The buffaloes under study showed 1st heat and uterine involution by about a month after calving.

Among the biochemical characteristics studied the serum AKP, PBI, total protein, free cholesterol, calcium, inorganic phosphorus and iron were found to be associated with the advent of parturition. Magnesium showed an increase around calving. Towards 1st postpartum heat and completion of uterine involution the serum PBI, total and ester cholesterol, inorganic phosphorus, iron and copper tended to increase. The enzymes AKP and peroxidase showed tendency for low levels towards follicular development and 1st heat. The serum total protein remained constant. Serum chloride showed low levels towards 1st postpartum heat. Higher level of magnesium in circulation might affect uterine involution.

Significant parity difference was found in serum AKP, total cholesterol, inorganic phosphorus, magnesium, chloride and copper.



The serum oestradiol—17B increased during the last stages of pregnancy and decrease was found a day after calving. The serum progesterone decreased before calving and further declined after calving. The higher level of oestradiol—17B and progesterone found during postpartum period appears to facilitate early

postpartum heat and uterine involution.

Reproductive efficiency with a calving interval of about 13 to 14 months is possible in Surti buffaloes. Looking to the performance, the estimates on the characteristics studied can be considered as good norms to judge the reproductive efficiency.



## **“Blood Serum Profile in Pregnant Buffalo (Surti Breed) During Different Stages of Pregnancy”**

*Name of Student* M.M. PATHAK

*Name of Advisor* Dr. K. JANAKIRAMAN

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Twentyfour pregnancies, from fertile heat to parturition have been covered for this study using Surti (Breed) buffaloes. Eleven biochemical characteristics and two steroid hormones have been estimated. Each characteristic was estimated using 672 samples and the hormones were estimated from the peripheral blood serum involving second pregnancy only, while rest covered 1 to 3 pregnancies. Blood sampling was done tapping the jugular vein.

Results indicated an increase in protein, protein bound iodine (PBI), alkaline phosphatase (AKP), peroxidase, cholesterol, phosphorus, progesterone and oestradiol-17 $\beta$  and decrease in calcium, iron, copper, chloride and magnesium in pregnancy compared to non-pregnant cycling animals.

Most of the estimates showed changes in the dam's circulation in the early stage (fertile heat to 50 days) and about a month before parturition. Alkaline phosphatase showed a decreasing trend toward parturition which is so glaring that it may be used to predict impending parturition. Serum protein started falling in the circulation 15 days prior to parturition, the fall in the PBI was greater in buffaloes towards parturition.

As far as minerals are concerned calcium, phosphorus, copper and iron attracted our attention. Circulating level of iron was less in pregnancy. Copper maintained an elevated plateau from 65 to 95 days of gestation which is known to be involved in the development of brain and nervous system. Estimated

values of phosphorus in the circulation was higher than that of cattle and though calcium and phosphorus fluctuated in their absolute values, a fairly constant ratio (P:Ca) was maintained (1:1.15 to 1:1.68 early to late pregnancy).

A preovulatory rise in progesterone was noticed. It reached peak on Day 15 of breeding and then fell down to one third. Again it increased five days antepartum and decreased appreciably 2 hr. prior to parturition. Increment in oestradiol-17 $\beta$  was noted on 3rd day and it decreased around Day 10, facilitating the probable events of egg transport and implantation. Serum oestradiol-17 $\beta$  from 25 days antepartum increased steadily till parturition. Towards term protein in the dam's circulation declined, it further declined after parturition probably due to its utilization in colostrum synthesis.

A sequential estimate over stages of pregnancy and also for successive three pregnancies was done. This is to map out the entire profile of a characteristic from fertile heat to 2 hr. postpartum. These data which are not hitherto available, fix the norms for normal pregnancy maintenance and parturition. This will also provide an index to examine deviations in situations, wherein a normal pregnancy—maintenance is threatened or complications arise at the delivery of foetus. The data will contribute a noteworthy addition in the field of buffalo reproduction and will be handy for researchers and clinicians.



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A special section is devoted to farm news in respect of progressive achievement in breeding efficiency and notification of valuable strains. The journal will be published in the months of August and February.

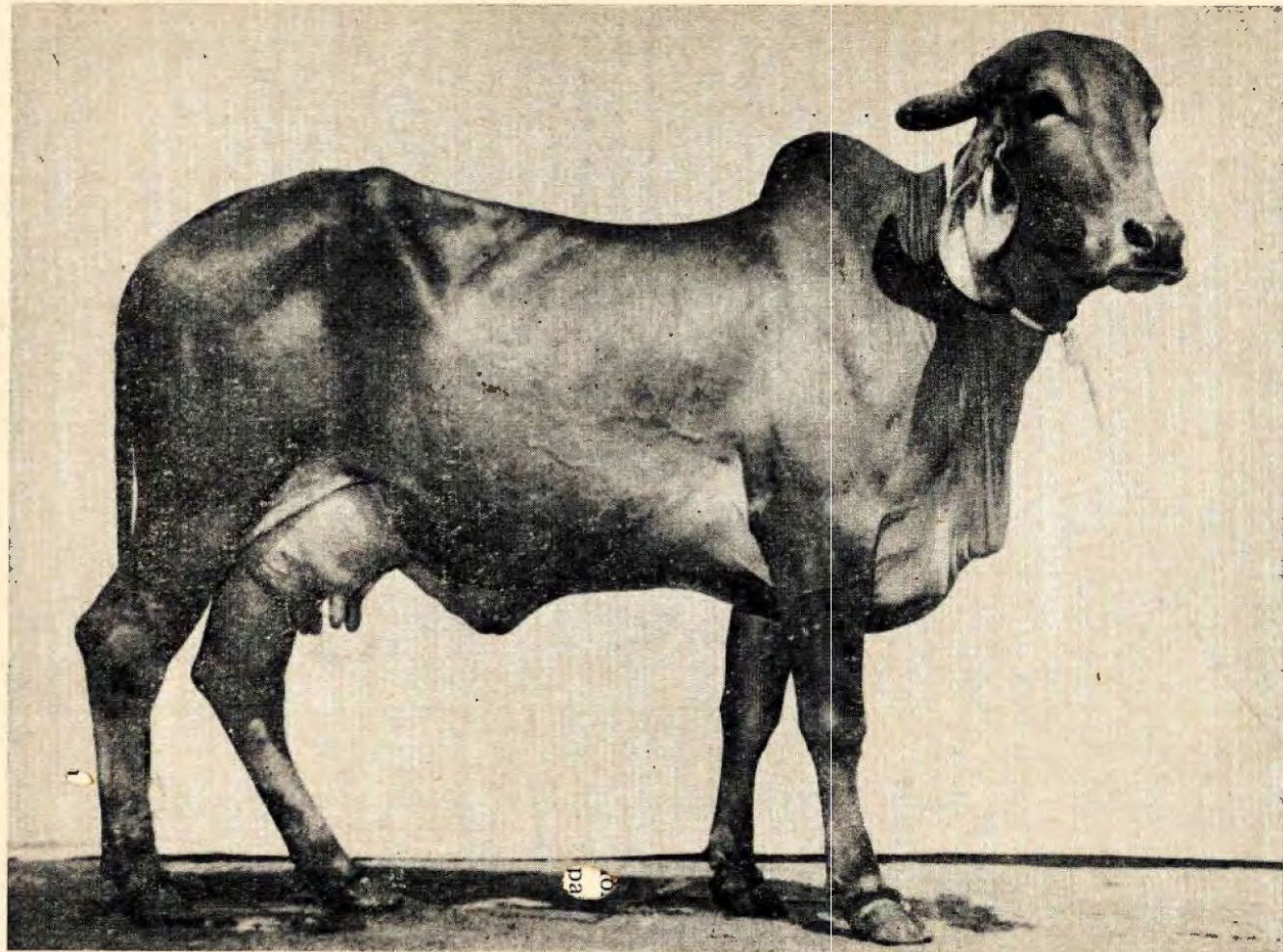
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Cow "CHANDAN" of Bombay Panjrapole



'A Milk Fountain'



## FARM NEWS

I.J.A.R. 4 : 1 : 99

### “CHANDAN”—A Milk Fountain

D.P. VELHANKAR

Bombay Veterinary College, Parel, Bombay-400 012.

Bombay Panjrapole is a leading institution engaged in maintaining the Gir germ plasm in the country. The Gir has proved to be the best milch animal under Bombay conditions and in Maharashtra as well. Very meticulous selection for better yielding strains coupled with adequate feeding and management practices has resulted in increase of average herd production. The average milk production per cow per day ( $n=147$ ) comes to 6.9 litres. Around 5 % cows yield 2300 liters whereas many have reached a level of 3700 liters in a lactation. This has been possible due to strenuous efforts for last several years.

The aim of the present communication is to place on record an unique performance by one of the cows of the Bombay Panjrapole viz. The cow named *Chandan*, (Vide-Photograph).

Chandan (Ear Tag No. 653/70, Brand No. 733) is a pure Gir animal born on 3rd February 1970. Born to sire '*Lalit*' and dam '*Majethi*' (5223 lg/305 days),

has a red brown colour and very majestic in appearance. Her lactation-wise performance is presented below:

During the state level milk yield competition held on 1.1.1981, Chandan recorded the maximum yield of 33.3 litres in 24 hours. She ranked first and was therefore awarded the first cash prize of Rs. 1000/- She was also adjudged as the best Gir animal on All India basis and bagged the first cash prize of Rs. 2000/- This is creditable not only to the Institution but to the State and the Nation at large.

The efforts do not end here alone. The institution undertakes research works in collaboration with recognised institutions. It also actively participates in extension education of Adivasis and marginal farmers in breeding, and management practices. The institution also distributes animals of various categories for improvement of local stock thus propagating the pure Gir germ plasm. The 'Bombay Panjrapole' has now become a nucleus centre for availability of pure Gir animals.

Lactation No.	Date of Calving	Sex of Calf	Total Milk Yield/Days	Intercalving Period-Days	Remarks
First	22.12.1974	Female	3605/336	—	
Second	9.3.1976	Female	2666/253	442	
Third	28.1.1977	Female	3680/364	325	
Fourth	19.7.1978	Male	2892.285	527	
Fifth	5.8.1979	Male	3455/270	382	
Sixth	17.8.1980	Female	3060/283	377	
Seventh	7.7.1981	Male	891/78	324	

The age at First Calving: 3 Yrs 11 months. Av. Intercalving period: 397.83 days.



## ISSAR NEWS

Recommendations made at the plenary session of All India Symposium on Recent Research Trends in Factors Influencing Fertility in Livestock held on 7th, 8th and 9th March, 1983 at the College of Vety. Science, Haryana Agricultural University, Hissar.

The Indian Society for the study of Animal Reproduction (ISSAR) in association with the Haryana Agri. University, Hissar and the Indian Council of Agri. Research, organised the All India Symposium at the Dept. of Gynaecology & Obstetrics, College of Veterinary Sciences, H.A.U. Hissar from 7th to 9th March, 83. The symposium was attended by 123 scientist delegates in the field of Animal Reproduction from various Agril. Universities, National Institutes, State Department of Animal Husbandry, Milk Federations, Insurances companies, Pharmaceuticals and Remount Veterinary Corps.

The Chief Guest Dr. R.M. Acharya, Dy. Director General (Animal Sciences), I.C.A.R. New Delhi in his key note address emphasised the need to give importance mainly on the following research: Nutrition in relation to reproduction; immunological aspects of fertility, embryo transfer programme; enhancing the fertility of male and female farm animals.

The following topics were discussed at various sessions of the symposium.

1. Improving reproductive potential of farm animals.
2. Reproductive failures and disorders.
3. Man, management and fertility.

In all, there were 106 papers on the above topics which were presented in 6 technical sessions. The abstracts of

relevant papers have been published in the Feb. 1983 issue of the Indian Journal of Animal Reproduction.

In addition to above there were 3 Panel discussions on the following subjects.

1. Progress through year in reproduction of cattle and buffaloes as influenced by Artificial Insemination.
2. Problems of gynaecological and obstetrical importance in livestock.
3. Problems concerning teaching and research in the subject of Veterinary Gynaecology & Obstetrics.

The symposium was a grand success. ISSAR. extends sincere thanks to the Organising Secretary, Dr. R.C. Gupta and his team for helping in organising this symposium at Hissar.

### *Dr. T.N. Khaladkar Prizes:*

Two prizes (Rs. 251/- each) have been instituted by Shri C. M. Ketkar of Poona in memory of late Dr. T.N. Khaladkar, Ex. Prof. of Veterinary Science, College of Agri. Poona.

The articles entitled "Cytogenetic studies of Murrah buffs. bulls" by A.K. Sharma, N.K. Vijay kumar, S.K. Verma, S.K. Khar, R.C. Gupta and N.K. Khurana and (2) "Compatibility between serum progesterone profile and clinical/rectal palpation findings in normal cycling and



suboestrous buffaloes" by F.S. Chauvan, R.D. Sharma, and G.B. Singh, were adjudged as winners of the prizes by the special committee. The prizes were distributed by the chief guest Dr. R.M. Acharya, Dy Director General ICAR. at the inaugural function on 7.3.83.

#### *ISSAR Fellowship Awards:*

The Fellowship of the Indian Society for the study of Animal Reproduction was bestowed upon the following dignitaries for their meritorious contribution in the field of Animal Reproduction.

Brig. Brij. Chandra, Dr. S.B. Kodagali, Dr. R.C. Gupta and Dr. B.R. Deshpande received the ISSAR. Fellowship Award at

the hands of Dr. R.M. Acharya, Dy. Director General ICAR. at the inaugural function of the symposium on 7.3.1983.

#### *The Indian Journal of Animal Reproduction*

It is a matter of satisfaction that ISSAR, has been successful in brining three issues—Decc. 1981, August, 1982 and Feb. 83 issues of the Indian Journal of Animal Reproduction. We express our thanks to the ISSAR. members, advertisers, ICAR. New Delhi and well wishers for their help and encouragement.

DR. B.R. DESHPANDE

*Hon. Secretary, ISSAR.*

### **Notification**

#### **Nils Lagerlof Memorial Award-1982**

The Indian Society for the study of Animal Reproduction is pleased to invite research/clinical articles on the subject of Animal Reproduction, published by Indian Authors in any of the journals during January to December, 1982, for consideration of the Nils Lagerlof Memorial Award for the year 1982.

Four copies of the reprints of the articles should be sent by the author to the Hon. Secretary, ISSAR. C/o Dept. of

Animal Reproduction, Bombay veterinary College, Parel, Bombay-400 012. The articles should reach the Hon. Secretary, ISSAR. latest by 31st August, 1983. The award is proposed to be presented at the inaugural function of the All India symposium on Animal Reproduction to be held in 1984.

Dr. B.R. DESHPANDE

*Hon. Secretary, ISSAR.*



# Indian Society for the Study of Animal Reproduction

Report on the All India Symposium on "Research Trends in Factors Influencing in Livestock" held at Haryana Agri. University Hissar on 7th, 8th and 9th March, 1983.

## Recommendations:

1. Research may be initiated for early detection of pregnancy using immuno-assay and enzyme assay procedures.
2. Investigations on functional and managemental causes of impaired reproduction should be taken-up simultaneous to the treatment of reproductive disorders.
3. With the increased scientific investigations involving hormonal studies, standardization of assay procedures in different laboratories be initiated.
4. To have better interpretative research for studies in the area of endocrinology and to compare the research results obtained in different laboratories, a few centres (Labs.) may be identified which will serve as nucleus quality control labs.
5. Research in the immuno reproduction-aspects for augmenting fertility may be taken-up.
6. Endocrine conditions leading to embryonic losses should be explored and investigated.
7. As sufficient data on profiles are available the possibility of extending it to explore the field situations and developing clinical and therapeutic applications based on data obtained be initiated. Where possible feed back from the field has to be made a routine.
8. ICAR may support few selected centres to produce required quality of antisera against specific hormones. Once such a facility is established, these products should be made available to the other laboratories free of cost for undertaking hormonal assay work.
9. ICAR /Govt may be approached with a request to establish Institute for Research in Animal Reproduction.
10. Where facilities exist, antibiotic sensitivity tests be carried out before treating genital infections. Facilities be created for such sensitivity tests at all levels.
11. Wherever indigenous drug preparations are available for reproductive disorders these may be given a fair trial. Laboratory investigations on the mechanism of action of new compounds/formulations should be taken up simultaneously.
12. State level laboratories be developed to undertake detailed andrological investigations including chromosomal aberrations for each breeding bull intended to be introduced for breeding purposes.
13. Refresher Courses be organised for inservice veterinarians and Stockman. Training programmes for Farm Managers and Farmers be also organised at frequent intervals in all the States.
14. Research on Embryo Transfer in various species of farm animals be intensified.
15. The ICAR has prepared & recommended a model syllabus in the subject of Animal Reproduction. Majority of Agri. Universities have adopted this model. In those Agri. Universities where the model syllabus is not followed in toto, should be requested to implement the same.



16. In order to intensify the practical training the ratio; between staff and students be 1:8 as is recommended by FAO.

17. ISSAR gratefully acknowledges the financial assistance of Rs. 5000/- received

from The ICAR New Delhi for holding the 3rd Symposium at Bombay and 4th symposium at Hissar.

(DR. B.R. DESHPANDE)

*Hon. Secretary, ISSAR*

## Capsules in Veterinary Practice

Institute for Research and Development of Dairy Cattle, B.G.M., in association with M.S.R. Foundation, Bombay and Bombay Veterinary College, have taken up a project to ascertain possibilities if capsules can be introduced in Veterinary practice for accuracy and

ease of administration of medicaments specially life saving and costly drugs like anthelmintics, antibiotics, feed supplements, minerals, trace elements etc. by oral and intrauterine routes. Experimental work is in progress with the aids extended by Associated Capsules Bombay.

## NOTIFICATION

The Indian Society for the Study of Animal Reproduction is pleased to announce that the Vth All India Symposium on Animal Reproduction is to be held at G.B. Pant University of Agriculture and Technology, Pantnagar on 27th, 28th & 29th February, 1984. All correspondence regarding Symposium should be addressed to Dr. S.N. Maurya, I/c. Department of Gynaecology and Obstetrics, College of Veterinary Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar, (Nainital) U.P. Pin code-263145, who is the Organising Secretary of the Symposium.

Articles to be presented at the forthcoming Symposium should be sent to the Organising Secretary, so as to reach him before 15th NOV. 1983.

Sd: DR. B.R. DESHPANDE

*Hon. Secretary, ISSAR.*



## Obituary

Late COL. DR. S.M. ISHAQUE

The members of the ISSAR. will be shocked to learn the sad demise of Col. Dr. S.M. Ishaque, Retired Dean and Prof. of Gynaecology and Obstetrics at the Ranchi Vety. College on 3rd April, 83 at Patna.

He was a founder member of ISSAR.

During the Second World War, he joined Netaji Subhas Chandra Bose's Indian National Army and as a patriot, he fought for the freedom of India. He was a prisoner of war and was released after the termination of the war. He joined the Patna Veterinary College and was posted as Prof. of Surgery. Considering his merits he was selected for FAO/SIDA International Course in Animal Reproduction at the Royal Veterinary College, Sweden where he obtained

F.R.V.C.S. in 1955. He toured the continental countries and acquired vast knowledge in education and research in the field of Reproduction in farm animals. He did substantial amount of research under the Cattle Sterility Scheme of I.C.A.R. and it is creditable for him to have placed on record the Trichomonas foetus infection for the first time in cattle. All credit goes to him for planning a Vety. College at Ranchi. In virtue of his meritorious services in the field of reproduction and education and research, the ISSAR. bestowed on him the Fellowship of the Society, in the year 1980. Even after his retirement, he was actively occupied in research in Ayurvedic Medicine.

Dr. Ishaque was a friend to one and all. His friends and admirers will ever remember him. May his soul rest in peace.



## 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:<br>Dept. of Gynaecology & Obstetrics<br>Gujarat Veterinary College, Anand |
| 2. Periodicity of Publication   | Bi-annual (FEBRUARY & AUGUST)   |
| 3. Printer's Name   | Prof. Dr. S.B. Kodagali   |
| Nationality   | Indian  |
| Address   | Gujarat Veterinary College, Anand-388001  |
| 4. Publishers Name  | Prof. Dr. S.B. Kodagali   |
| Nationality   | Indian  |
| Address   | Gujarat Veterinary College, Anand-388001  |
| 5. Editor's Name  | As Above  |
| Nationality   |   |
| Address   |   |
| Name 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<br>THE INDIAN SOCIETY FOR<br>THE STUDY OF<br>ANIMAL REPRODUCTION          |

Regd. No.: Bom. 253/78

Office:

Dept. of Animal Reproduction  
Bombay Veterinary College Parel,  
Bombay-400 012

I Prof. Dr. S.B. Kodagali, Editor of THE INDIAN JOURNAL OF ANIMAL REPRODUCTION hereby declare that the particulars given above are true to the best of my knowledge and belief.

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