

# THE INDIAN JOURNAL OF ANIMAL REPRODUCTION

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# EDITORIAL

A Joint Technical Seminar on "Recent Advances and incoming technologies in Livestock Development" organised by Institute National De La Recherche Agronomique (INRA), France and Bharatiya Agro-Industries Foundation (BAIF), India as a part of French Festival in India-1989 was held at BAIF, Pune from 11th to 13th February 1989. A distinguished gathering of Scientists from INRA, BAIF and several Indian organisations, Universities and important Departments of Govt. of India and ICAR participated in the Seminar. The technical recommendations made by the Scientific Experts at this Seminar pertaining to Animal Reproduction are given hereunder for the benefit of our esteemed members/readers:—

## **1. Frozen Semen Technology**

1. Artificial Insemination through the intervention of Frozen Semen Technology is the most powerful and practical means of transferring and disseminating high quality genes for massive campaigns for livestock breeding in India.

2. It was essential to enforce strict sanitary controls at the semen collection and A.I. centres, by routine diagnostic testing of herds.

3. Quality control of frozen semen involving tests for absence of pathogenic micro-organisms and viruses such as Brucella, IBR virus etc. should be made compulsory.

4. Techniques of Early Pregnancy Diagnosis and Oestrus Synchronisation should be applied in India for achieving rapid, planned and efficient implementation of cattle breeding programmes.

## **2. Embryo Transfer Technology**

1. Embryo Transfer and Multiple Ovulation and Embryo Transfer (MOET) technologies being important tools for improvement of exotic herds and rapid multiplication of high yielding dairy cattle, research work on these technologies should be initiated.

2. Sanitary measures for the donor animals and quality control of frozen semen and embryos must be strictly enforced so as to prevent passage of infectious agents through the embryos.

Padmashri Dr. Manibhai Desai President and Dr. D.S. Gorhe, Vice Executive President, BAIF deserve special congratulations for organising such an important Indo-French Seminar. We are thankful to them for inviting Dr. A.S. Kaikini, Editor IJAR to participate in this useful Seminar.

We invite the kind attention of our readers for the forthcoming National Symposium on "Applied Reproduction in Farm Animals and the 8th National Convention of ISSAR to be held at Anand (Gujarat) between November 1989 to February 1990. Details are printed elsewhere in this issue which may please be perused.

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**From Editor's Pen**

**From Secretary's Desk**

**ISSAR News**

### **First Announcement**

#### **NATIONAL SYMPOSIUM ON "APPLIED REPRODUCTION IN FARM ANIMALS" AND THE VIIIth NATIONAL CONVENTION OF INDIAN SOCIETY FOR THE STUDY OF ANIMAL REPRODUCTION. (ISSAR).**

The Gujarat chapter of the Indian Society for the Study of Animal Reproduction (ISSAR), in association with the Gujarat Agricultural University will be holding a National Symposium on "Applied Reproduction in Farm Animals" and the National Convention of ISSAR at ANAND (Gujarat) between November, '89 to February, '90.

**Tentative Dates: 10-11-12 November, 1989**

Scientists/Researchers interested to participate should send their Registration fees by Bank Demand Draft, drawn in favour of "Treasurer ISSAR (Gujarat Chapter) Anand" to the Organising Secretary, before 30th September '89.

Delegate Fee Rs. 250/-  
Accompanying adult Rs. 150/-  
Accompanying child Rs. 50/-

For further details, please contact:—

**Dr. V.M. Mehta**

Organising Secretary,

National Symposium on "Applied Reproduction in Farm Animals"

Reproductive Biology Research Unit, Gujarat Agricultural University, ANAND, 388 110.

Phone : 21666 Ext. 258



## In Memorium



Dr. M.R. Marathe

We are deeply grieved to inform about the sad demise of Dr. M.R. Marathe, GBVC, FRVCS (Sweden), Fellow ISSAR a well known Veterinary Scientist of BAIF at Pune on 18th February 1989.

Dr. Marathe was Chief Veterinary Officer of Aarey Milk Colony, Goregaon, Bombay, right from its inception and possessed rich experience in all aspects of Buffalo Production and Management. The late Professor Nils Lagerlof, FAO Expert in Animal Reproduction during his visit to Aarey Milk Colony way back in 1953 was deeply impressed by Dr. Marathe whom he termed as "Buffalo Man of India".

Dr. Marathe later joined BAIF, Uruli-Kanchan and contributed to its growth and prosperity based on livestock economy. His passing away is indeed an irreparable loss.

We extend our heartfelt condolences to the bereaved family members including BAIF family.

## ERRATA

The following errors in IJAR Vol. 9, No. 2, December 1988 may please be corrected. Inconvenience caused is regretted.

Sr. No.	Item	Error	Correction
1.	Contents Sr. No. 20	M.P. Singh	M.P. Sinha
2.	Article 19 Page No. 131	S.K. Singh	B.K. Singh
3.	Article 20 Page No. 134	M.P. Singh	M.P. Sinha



## C.R. SANE ORATION FUND AN APPEAL

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

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

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

A. RAMAMOHANA RAO  
PRESIDENT — ISSAR

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## Age At Maturity In Sahiwal Cross-bred Heifers With Three Levels Of Exotic Inheritance\*

A.W. DESHMUKH<sup>1</sup> and A.S. KAIKINI<sup>2</sup>

Department of Gynaecology and Obstetrics, Nagpur Veterinary College, Seminary Hills, Nagpur-440 006

### ABSTRACT

Studies on age at maturity were carried out on 133 Sahiwal crossbred heifers with three levels of Jersey exotic inheritance. The results indicated that J × S half-breds had better reproductive performance, regarding age at maturity, as compared to J × S with 62.5 per cent and 75 per cent level of exotic inheritance. The effect of period and farm on age at maturity had highly significant effect on age at maturity.

\* \* \*

The economic loss due to delayed maturity may be minimized if its causes are investigated. The present attempt deals with study of age at maturity in Jersey × Sahiwal cross-bred heifers with three varying levels of exotic inheritance.

### Materials and Methods

The data on Sahiwal cross-bred heifers with three levels of Jersey exotic inheritance 50 per cent, 62.5 per cent and 75 per cent, maintained at Cattle Breeding Farms of Veterinary College and Agriculture College, Nagpur were studied. The data of last 15 years (1971 to 1986) was grouped in three periods each consisting of 5 years each, P<sub>1</sub> (1971-1976); P<sub>2</sub> (1977-1981) and P<sub>3</sub> (1982-1986). A total of 133 cross-bred females studied were divided in three groups as per their exotic inheritance, thus: (1) 50 per cent - 81 animals (2) 62.5 per

cent - 25 animals and (3) 75 per cent - 27 animals.

The data was scrutinized to study the effect of level of exotic inheritance, the period and the farm in Sahiwal cross-breds on their age at maturity by applying least square analysis of variance design.

### Results and Discussion

The over-all average age at maturity for J × S cross-breds with 50 per cent, 62.5 per cent and 75 per cent level of exotic inheritance was found to be 825.93±33.70, 905.60±57.60 and 875.62±62.19 days respectively.

The average age at maturity for all the three periods, irrespective of inheritance was found to be 588.20±45.77; 761.27±41.83 and 984.10±33.06 days in J × S cross-bred heifers. The average period for age at maturity in the Agriculture College Dairy Farm and Veterinary College Farm was found to be 950.74±45.14 and 796.48±31.06 days respectively.

The least square analysis of variance to test the differences among means for these three levels of Jersey inheritance revealed highly significant difference ( $P < 0.01$ ). The results indicated highest average age at maturity for 62.5 per cent exotic inheritance level (905.60±57.30 days) followed by 75 per cent exotic inheritance level (875.62±62.19

\*Part of the Ph.D. Thesis submitted to the Punjab Agricultural University (PKV), Akola-444104.  
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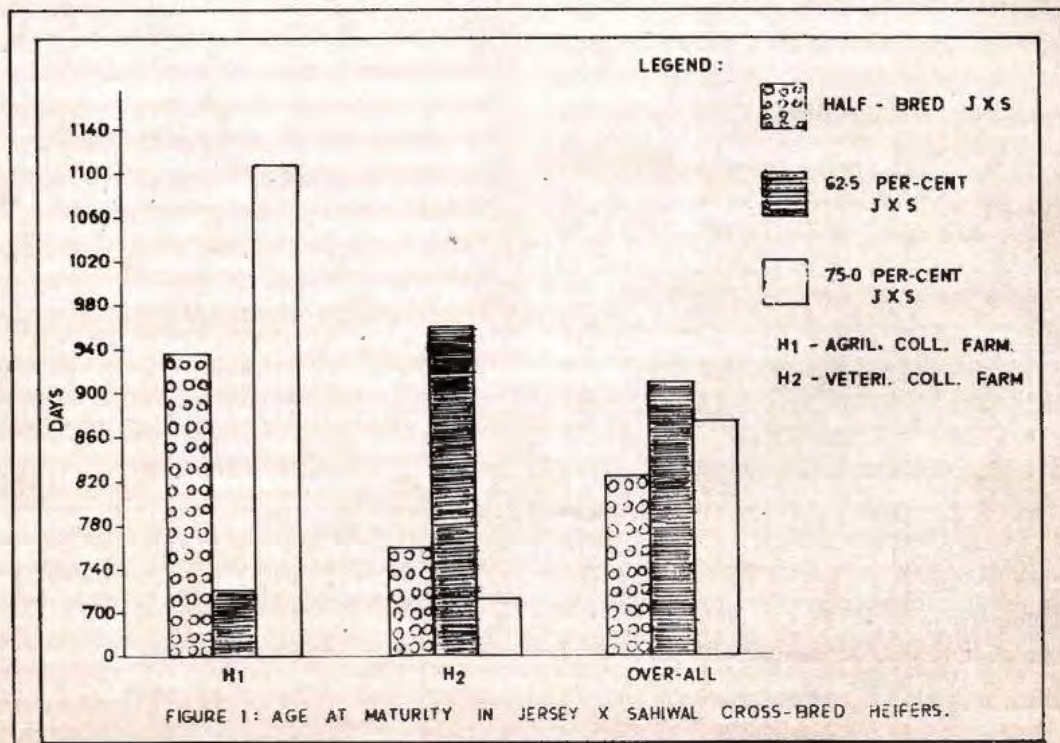
days) with the lowest average age at maturity in half-breds ( $825.93 \pm 33.70$  days).

The present findings indicated superiority of half-breds over the two other levels of exotic inheritance. Kale (1978) observed the same trend in respect of Sahiwal  $\times$  HF cross-bred heifers with three levels of exotic inheritance. However these differ from Gangwar *et al* (1973) who recorded increase in values from 62.5 per cent to 75 per cent exotic inheritance levels, and McDowell *et al* (1976) and Goel and Singh (1986) who recorded decrease in values from 50 per cent to 75 per cent exotic inheritance for age of maturity. Manickam *et al* (1978) found  $755 \pm 37.3$  days age at maturity in Jersey  $\times$  Sindhi half-breds. This is in partial agreement with the present findings in half-breds ( $825.93 \pm 33.70$  days).

There was significant difference for average age at maturity between the three periods studied. Highest average age (984.39

days) at maturity was recorded for  $P_3$  period as compared to lowest (588.20 days) in  $P_1$  period. The lowest age at maturity of cross-bred heifers in first ( $P_1$ ) period could be attributed to ideal managerial conditions adapted when the cross-breeding programme of Sahiwal  $\times$  Jersey was implemented during this initial period. The delayed age at maturity in subsequent periods might be due to deviations in managerial practices. Similar findings of variation due to period effect were also reported by Rajan (1977).

There was significant difference for average age at maturity between the two farms studied for J  $\times$  S cross-bred heifers. The interaction between levels of inheritance and periods, levels of inheritance and farms, farms and periods studied was non-significant. This is indicative of the fact that the J  $\times$  S cross-breds with three levels of inheritance did not interact with the periods, as well as farms.





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## Effect Of Beta-Carotene On Certain Aspects Of Reproduction In Dairy Heifers

### 1. Pattern Of Oestrus

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#### ABSTRACT

Twenty crossbred heifers in the age group of 2-3.5 years and body weight varying from 130-190 kg were used in the study. All the heifers were kept on a  $\beta$ -carotene free basic ration consisting of concentrate @ 3 kg per heifer per day and paddy straw *ad libitum*. Each heifer in the control group received a daily supplement of 60,000 I.U. vitamin A in their feed, while in the experimental group each heifer was given 20,000 I.U. vitamin A plus 100 mg  $\beta$ -carotene daily. The incidence of pronounced oestrus was 23.33% and 78.57% and that of weak oestrus was 76.67% and 21.43% in the control and experimental groups respectively. The mean duration of oestrus in the control group ( $22.15 \pm 1.11$

hours) was significantly ( $P < 0.01$ ) higher than in the experimental group ( $17.90 \pm 0.48$  hours)

\* \* \*

Green fodder does not contain vitamin A, but only its precursor  $\beta$ -carotene, which is converted to vitamin A in the animal system. In India, there is acute scarcity of green fodder except during monsoon. The mainstay of cattle feed in India is dry fodder like paddy straw, wheat straw and oat straw. Cattle fed on low carotene ration are reported to have lower level of carotene in blood plasma (Sen and Rai Sarkar, 1942) and impairment of reproductive functions (Meyer *et al*, 1975 and Lotthammer *et al*, 1978). The present study was aimed to know the effect of  $\beta$ -carotene on the pattern of oestrus in dairy heifers.

<sup>1</sup>Associate Dean, Lakhimpur College of Veterinary Science, Assam Agricultural University, Azad, North Lakhimpur-787001



## Materials and Methods

Twenty crossbred heifers of Instructional Livestock Farm, College of Veterinary Science, AAU, Khanapara, Assam in the age group of 2-3.5 years and body weight varying from 130-190 kg were used for the study. The animals were grouped into control and experimental groups, comprising ten heifers, each.

All the heifers were kept on a  $\beta$ -carotene free basic ration which consisted of concentrates and paddy straw. The concentrate was fed to heifers @ 3 kg heifer per day and the paddy straw was given *ad libitum*. Each heifer in the control group was given a daily supplement of 60,000 I.U. vitamin A (Vitablend, Glaxo) but in the experimental group each heifer was given 20,000 I.U. vitamin A plus 100 mg  $\beta$ -carotene daily. Dry Beta-carotene (Roche) 10% water soluble. (F-Hoffmann La Roche & Co. Ltd., Basle, Switzerland). As the conversion rate of  $\beta$ -carotene to vitamin A is 1:400 (Hemken and Bremel, 1982), the total equivalent vitamin A supplement in the heifers of both the groups was the same. Other managerial conditions were uniform.

A total of 30 and 28 oestruses were studied in the control and experimental groups respectively. The heifers were observed at an interval of 6 hours for detection of oestrus. The intensity of oestrus was recorded as pronounced or weak on the basis of external and behavioural symptoms and gynaecological status of genital organs. The duration of oestrus was recorded as the period from the time the oestrus symptoms were first observed till these subsided.

## Results and Discussion

The incidence of weak oestrus was observed to be higher in the control group (76.67%) while that of pronounced oestrus was higher (78.57%) in the experimental group (Table 1). This observation is in agreement with that of earlier workers (Ahlsweide *et al*, 1976; Lotthammer, 1979; Heinz and Herzog, 1982; Bonsembiante *et al*, 1983 and Tekpatey, 1985). The exhibition of weak oestrus as a result of  $\beta$ -carotene deficiency observed in the present study might be due to the depressed production of steroid hormone in the affected animals (Jackson *et al*, 1981).

**Table 1: Intensity and duration of oestrus in heifers.**

Groups	No. of animals	Intensity of oestrus		Duration of oestrus (hours)	
		Pronounced (%)	Weak (%)	Range	Mean $\pm$ SE
Control	10	23.33(7)	76.67(23)	10.50-40.0	22.15 $\pm$ 1.11
Experimental	10	78.57(22)	21.43(6)	14.0-23.0	17.90 $\pm$ 0.48

Figures in Parentheses indicate number of oestruses

\*\*P < 0.01

The duration of oestrus was significantly longer ( $P < 0.01$ ) in the control group (Table 1). This finding corroborates with that of

Friesecke (1978) and Lotthammer (1979). On the contrary, Folman *et al* (1979) and Ducker *et al* (1984) did not find significant difference



in the duration of oestrus in  $\beta$ -carotene supplemented and unsupplemented cows. The duration of oestrus in the experimental heifers was observed to be closer to the normal duration of oestrus in cattle as observed by earlier workers (Trimberger, 1948; Mathai and Raja, 1978). But the range of oestrus in the control heifers was observed to be wider than normal range. The longer duration of oestrus observed in the control heifers might be due to a disturbance in the production of

FSH and LH, resulting in slower final growth of the follicle.

In the present study, the  $\beta$ -carotene deficiency was observed to impair the pattern of oestrus in dairy heifers. The present findings, based on a smaller number of animals, indicate the possibility of mitigating the impairment of reproduction in cattle by supplementing  $\beta$ -carotene in concentrates and feeding paddy straw during the period of green fodder scarcity.

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## Serum FSH And Oestradiol — $17\beta$ Concentrations In Postpartum Anoestrous Crossbred Cattle\*

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### ABSTRACT

FSH and Oestradiol -  $17\beta$  concentrations in three Gir  $\times$  HF anoestrous cows were estimated by Radioimmunoassay (RIA), intermittently over a period of 24 days. Estimated FSH concentrations were quite basal and differed widely between different days, although statistically they did not differ significantly. An overall average FSH level was found to be  $22.68 \pm 3.34$  ng/ml of serum. The recorded Oestradiol -  $17\beta$  levels were also quite basal, the differences between them on different days being non-significant. An overall Oestradiol -  $17\beta$  level was found to be  $1.72 \pm 0.17$  pg/ml of serum.

\* \* \*

There is little doubt that the inhibitory effects of the factors such as nutritional status, season, suckling and lactation on the reproductive system are mediated by endocrine system and probably via a common final mechanism (Peters and Lamming, 1984). Hence in the present study, it was decided to investigate the endocrine picture of anoestrous cows as regards FSH and Oestradiol -  $17\beta$ . Barnes *et al* (1980) observed the average plasma concentration of FSH and Oestradiol -  $17\beta$  as 19.0 ng/ml and 4.3 pg/ml, respectively, in five anoestrous

dairy cows. In India, Kodagali (1981) and Pargaonkar *et al* (1983) reported average FSH concentrations in anoestrous cows as  $30.61 \pm 4.33$  and 32 ng/ml of serum, respectively.

### Materials and Methods

The present study was conducted on three postpartum anoestrous Gir  $\times$  HF cows, belonging to Bombay Cow-Rakshak Mandali's Cattle Breeding Farm, Kandivili, Bombay, during May 1987. The three cows viz., Shila ii, Harni and Kiran were in their 11, 11 & 1 lactations and anoestrous for 248, 461 and 136 days after their calvings, respectively. They were apparently healthy and were clinically free from any reproductive pathology.

*Collection of blood samples and separation of serum:* Blood samples were collected consecutively for four days. Thereafter, blood samples were taken on days, 7, 14, 18, 19, 20, 21, 22, 23 and 24 from the day of first sample. Each sample was collected in duplicate. Blood samples after collection were kept for 24 hours in refrigerator for the separation of serum. Serum was then collected in sterile vials, to which a drop of Merthiolate solution (1:1000 w/v) was added per ml of the serum. After

\*Forms a part of the M.V.Sc. thesis of the senior author submitted to Konkan Krishi Vidyapeeth, Dapoli.

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sealing, these vials were stored in deep freeze at  $-20^{\circ}\text{C}$  till the estimations of FSH and Oestradiol -  $17\beta$  concentrations were carried out by RIA technique.

**RIA of FSH from serum samples:** To measure the circulating levels of FSH, RIA was conducted by double antibody technique of Midgley (1966), using highly purified ovine FSH (NIAMMD - oFSH - I - 1) for radioiodination and anti-ovine FSH serum (NIAMMD - anti- oFSH - 1) for binding studies, both of which were donated by National Institute of Arthritis, Metabolism and Digestive Diseases, U.S.A.

Radioiodination of oFSH was carried out as per the methods of Greenwood *et al* (1963) as modified by Midgley (1966). The assay was developed with a sensitivity of 0.31 ng of bovine FSH (USDA - bFSH - B - 1) per tube and was capable of quantifying FSH in 100  $\mu\text{l}$  of serum. Anti-ovine-FSH-serum was used at a final dilution of 1:50,000, after determining its titre through binding studies. A standard dose - response curve was prepared by

plotting the corresponding percent bound (B/Bo) against the standard doses on Logit - log paper. The concentrations of FSH in serum samples were then determined from the standard curve. All the collected samples were subjected to FSH estimation.

**RIA of Oestradiol -  $17\beta$  from serum samples:** The serum samples collected on days 0, 7, 14, 18 and 24 from the day of first sample were subjected to quantitative determination of Oestradiol -  $17\beta$  using Biodata (Italy) Estradiol-125 I Ter kit. The kit has been designed for the direct measurement of Oestradiol -  $17\beta$ , without any pre-treatment of the sample. A standard curve from 10 pg to 500 pg/ml was plotted, on which the Oestradiol -  $17\beta$  concentration present in the sample was directly read. The test required only 50  $\mu\text{l}$  of the serum. All the samples were assayed in a single assay to avoid inter-assay variation.

The data was analysed as per the methods described by Snedecor and Cochran (1967).

**Table 1 : Mean Serum FSH and Oestradiol Concentration on different days**

Days	0	1	2	3	7	14	18	19	20	21	22	23	24
FSH	34.00	20.00	19.26	23.00	14.26	42.33	13.00	20.33	20.76	46.66	12.00	16.26	13.00
conc.	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
(ng/ml)	10.01	8.32	14.46	9.29	6.29	5.78	6.11	3.38	18.61	8.96	0.00	12.89	8.18
Oes- $17\beta$	2.06	—	—	—	1.20	1.43	2.03	—	—	—	—	—	1.90
conc.	$\pm$				$\pm$	$\pm$	$\pm$						$\pm$
(pg/ml)	0.52				0.40	0.32	0.62						0.15

Average FSH Conc. =  $22.68 \pm 3.13$ . Average Oestradiol  $17\beta$  Conc. =  $1.72 \pm 0.17$



**Table 2: Analysis of Variance of mean FSH and Oestradiol-17 $\beta$   
levels in Post-partum anoestrous cows**

Source of Variation	FSH			Oestradiol 17 $\beta$		
	D.F.	M.S.	'F' Value	D.F.	M.S.	'F' Value
Between days	12	382.81	1.329 NS	4	0.45	0.793 NS
Error	26	287.88		10	0.57	
Total	38			14		

NS — Non-significant

### Results and Discussion

The serum FSH concentrations recorded in the present study were quite basal and differed widely between days, although, statistically these differences were non-significant. An overall average FSH concentration was found to be  $22.68 \pm 3.13$  (Table 1).

The pattern of FSH concentrations in post-partum anoestrous cows, recorded in the present study agrees well with the findings of Schams *et al* (1978), who found widely differing peak values and irregular spikings of FSH levels in post-partum anoestrous cows.

Base values throughout the anoestrous period of cows observed in the present investigation correspond with those recorded by Schams *et al* (1978) and Barnes *et al* (1980). The pattern of FSH concentrations as well as observed levels in anoestrous cows in the present study are also in agreement with those recorded by Kodagali (1981) and Pargaonkar *et al* (1983), who reported average FSH concentrations in anoestrous cows as  $30.61 \pm 4.33$  and  $32$  ng/ml of serum, respectively. FSH levels were found to fluctuate around  $40$  ng/ml in anoestrous cows by Bolt and Rollins (1983).

The mean Oestradiol - 17 $\beta$  levels in post-partum anoestrous cows on different days,

recorded in the present investigation were quite basal, the differences between them being non-significant. An overall Oestradiol - 17 $\beta$  level was  $1.72 \pm 0.19$  pg/ml of serum.

The Oestradiol - 17 $\beta$  levels observed in the present study are comparable to those found by Carruthers *et al* (1980), who reported the mean Oestradiol - 17 $\beta$  concentrations as  $2.9 \pm 0.3$  and  $3.0 \pm 0.4$  pg/ml of plasma in suckled and milked cows, respectively. In the study of Rawlings *et al* (1980), Oestradiol - 17 $\beta$  levels in 7 Hereford cows during post-partum anoestrous period fluctuated between  $0$  to  $20$  pg/ml of plasma. However the present findings deviate somewhat from those of Barnes *et al* (1980) and Humphrey *et al* (1983), who detected basal average concentrations of Oestradiol - 17 $\beta$  in post-partum acyclic cows as  $4.3$  pg/ml of plasma and  $7 \pm 3$  pg/ml of serum, respectively. The deviation can well be attributed to breed and individual variations, time of sampling, storage of samples, method of Oestradiol - 17 $\beta$  estimations, purity and quality of chemicals used.

### Acknowledgements

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## Serum Progesterone Levels in Repeat Breeder Jersey Cows

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### ABSTRACT

The mean serum progesterone levels on various days of estrous cycle in repeat breeder, and non-repeat breeder Jersey cows varied between  $0.25 \pm 0.02$  and  $2.76 \pm 0.19$ , and  $0.38 \pm 0.04$  and  $5.16 \pm 0.35$  ng/ml, respectively.

Though the serum progesterone levels were lower in repeat breeder than that in the non repeat breeder Jersey cows on all the days of observation the differences were significant ( $P < 0.01$ ) on day 8, 13 and 16 post estrus.

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Répeat breeding in cattle has been recognised as a serious problem affecting the economy of dairy farmers. Though the specific causes of repeat breeding cannot be pin pointed, the abnormal levels of ovarian steroids contribute considerably to this disorder. The present study reports the serum progesterone levels in repeat breeder cattle.

### Materials and Methods

A total of 12 Jersey cows apparently in good health were included in this study. The animals were kept under uniform feeding and managerial practices at the College Instructional Livestock Farm, Khanapara, Guwahati, and divided into two groups. Group I : Repeat breeder cows: Six regularly cyclic cows having apparently normal genitalia and served by AI earlier for more than 3 services without conception. Group II : Control animals: Six cows with normal genitalia and pregnant with ooe to three AI.

Five blood samples were collected from each of the cows from jugular vein on day 1 of

oestrus and 4, 8, 13 and 16 days of the oestrus cycle. The serum was separated from the collected blood samples and stored at  $-20^{\circ}\text{C}$  till processed for progesterone. The serum progesterone was estimated by double antibody technique using kits procured from Leeco Diagnostics, Inc. 21705 Evergreen Southfield, Michigan 48075. The sensitivity of the assay was 0.1 - 1.5 ng/ml. Intra-assay C.V. was 7.5 per cent.

### Results and Discussion

Although there were individual variations in progesterone levels on all the days of observation, the mean serum progesterone levels continued to incline from day 1 till day 13 post estrus in both the groups of cows (Table 1). On day 16 post estrus, the mean serum progesterone levels in cows of both groups were comparatively lower than that on the 13th day post-estrus. The serum progesterone levels in normal cows during estrous cycle, reported earlier (Robertson, 1972; Ahmed *et al.* 1977) simulate the findings of the present study.

**Table 1: Serum progesterone level (ng/ml.) in repeat breeder and normal Jersey cows.**

Group of cows	Days of estrous cycle				
	1	4	8	13	16
I. Repeat breeder	$0.25 \pm 0.02$ (6)	$0.34 \pm 0.04$ (6)	$1.66 \pm 0.20$ (6)	$2.76 \pm 0.19$ (6)	$2.12 \pm 0.12$ (6)
II. Non-repeat breeder	$0.38 \pm 0.04$ (6)	$0.67 \pm 0.05$ (6)	$2.70 \pm 0.15$ (6)	$5.16 \pm 0.35$ (6)	$4.30 \pm 0.24$ (6)
't' value	0.103 NS	0.5066 NS	6.6115**	9.5770**	13.06**

\*\*  $P < 0.01$ ; NS Not significant.

Figures in parentheses indicate number of observations.

Though the serum progesterone levels in the repeat breeder cows were lower than that in the non repeat breeder cows on all the days of observation, the differences were significant ( $P < 0.01$ ) on 8th, 13th and 16th

days post-estrus. Agarwal *et al.* (1982) reported similar value of serum progesterone levels in normal and repeat breeding cows on day 1, 13 and 16 of the estrous cycle.



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## Immuno-electrophoretic Characteristics Of Seminal Antigens In Sindhi, Jersey And Cross-Bred Bulls\*

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### ABSTRACT

Immuno-electrophoresis of seminal plasma from 53 bulls resolved maximum number of 14, 10, 13 and 13 seminal antigens in Sindhi, Jersey, Sindhi × Jersey and Sindhi × Friesian bulls. There were three major antigen fractions common and identical to all breeds. The frequency of occurrence and location of other antigens varied among the local, exotic and cross bred bulls.

\* \* \*

Fractionation and characterisation of seminal antigens was attempted in many species. Sudarsanam (1983) identified 20 antigens in pooled buffalo semen. Kulkarni (1985) noted 4-6 seminal plasma proteins of the buffalo and cattle to be antigenically similar to their serum proteins. Human seminal plasma revealed minor but distinct inter individual differences in the antigenic

component (Defazio *et al.* 1969). In the present studies an attempt is made to characterise and identify the common and uncommon seminal antigens among Sindhi, Jersey and Cross bred bulls.

### Materials and Methods

53 reproductively healthy breeding bulls comprising of 13 Sindhi, 15 Jersey, 15 Sindhi × Jersey and 10 Sindhi × Friesian bulls were utilised. Semen collected by artificial vagina from these bulls was examined for normal spermatozoal motility, viability and concentration.

Sixteen adult male rabbits were utilised to prepare hyperimmune serum against Sindhi, Jersey and Cross-bred bulls semen as per the standard technique. The method described by Crowle (1961) was adopted to find out the immuno-electrophoretic pattern of seminal antigen. Individual bull seminal plasma was

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taken in the wells on either side of the trough and subjected to electrophoresis. Then the antisera of the corresponding breed was charged in the trough. The precipitin lines were studied after 72 hours.

### Results

The average number of seminal antigens identified in the whole semen of Sindhi, Jersey, Sindhi × Jersey and Sindhi × Friesian Jersey bulls were 12(9-14), 9(7-10), 11(8-13) and 10(9-13) respectively. However, any one sample showed no more than 9-10 antigens. Immune electrophoresis of pooled semen revealed fourteen seminal antigens (fig. 4a). Antigens 1, 3 and 7 were the most prominent seminal antigens which were deeply stained and also seen in all Sindhi bulls seminal plasma studied. The other antigens vary in their frequency of occurrence. Antigens 2, 5, 6, 8 were seen in nearly 50 per cent of the samples while antigens 4, 10, 12, 13 and 14 were faint and were noted only in 15 per cent of samples.



Fig. 1 Immunoelectrophoresis of Jersey Seminal Plasma

Immuno-electrophoresis of Jersey seminal plasma revealed slight difference from that of Sindhi (Fig. 1; 4b). Antigens 4, 8, 12, 13

FIGURE - 4  
COMPOSITE DIAGRAM OF ANTIGEN-ANTIBODY  
PRECIPITIN LINES RESOLVED BY IMMUNOELECTROPHORESIS

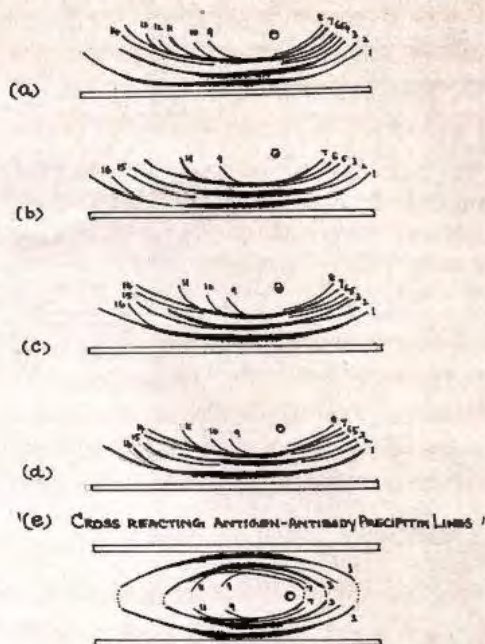


Fig.4 Composite Diagram of Antigen - Antibody Precipitin Lines resolved by Immunoelectrophoresis

- (a) Sindhi Seminal Plasma
- (b) Jersey Seminal Plasma
- (c) Sindhi x Jersey Seminal Plasma
- (d) Sindhi x Friesian Seminal Plasma
- (e) Cross reacting Antigen - Antibody Precipitin Lines

and 14 seen in Sindhi were absent in Jersey semen. However, two additional antigens were noticed (15 and 16). Though faint, they were seen in all the semen samples. The prominent deeply stained antigens 1, 3 and 7 were certainly seen in all semen samples studied. Antigens 2, 9 and 11 were observed in 66 per cent of samples, while antigens 5, 6 were seen only in 33 per cent of the samples.

Immuno-electrophoresis of the Sindhi × Jersey cross bred bulls seminal plasma revealed the presence of 13 antigens (Fig. 2a; 4c). The deeply stained antigens 1, 3 and 7 were distinctly seen in all the samples. Antigens 4, 12, 13 were absent but antigens 15 and 16



seen in Jersey were noticed even in Sindhi  $\times$  Jersey bulls semen. Antigens 2, 8, 9, 11, 14 and 15 were present in 66 per cent of the samples, while antigens 5, 6 and 10 were seen in only 33 per cent of the samples examined.

Immuno-electrophoresis of seminal plasma of Sindhi  $\times$  Friesian bulls revealed 13 antigens (Fig. 2b; 4d). The incidence and location of antigens were similar to Sindhi  $\times$  Jersey cross bred bulls semen. Only 1, 3 and 7 were deeply stained and were seen in 100 per cent of the samples examined. Antigens 2, 9, 11, 14 and 16 were seen in 80 per cent of the samples. Antigens 5, 6, 8 were observed in 50 per cent and antigen 10 were seen only in 30 percent of the samples.

To evaluate cross reacting and identical antigens between Sindhi, Jersey, Sindhi  $\times$  Jersey and Sindhi  $\times$  Friesian cross bred bulls, their semen was taken in the central well and after electrophoresis, the troughs on either side were charged with antisera of the same or of different breeds. It is evident from the immuno-electrophoretic resolution (Fig. 3a,



Fig. 2 (a) Immuno-electrophoresis of Sindhi  $\times$  Jersey Seminal Plasma  
(b) Immuno-electrophoresis of Sindhi  $\times$  Friesian Seminal Plasma

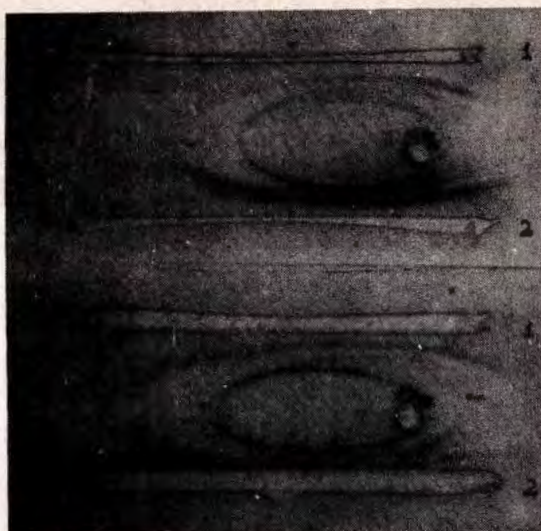


Fig. 3 Cross reactions of seminal antigens of different breeds.

- (a) Sindhi Seminal Plasma  
(1) Anti Sindhi Semen Serum  
(2) Anti Jersey Semen Serum
- (b) Sindhi Seminal Plasma  
(1) Anti Sindhi  $\times$  Jersey Semen Serum  
(2) Anti Sindhi  $\times$  Friesian Semen Serum

3b; 4c) that antigens 1, 3 and 7 were identical as they show union at the end in Sindhi, Jersey and cross bred bulls examined. Antigens 9 and 11 were partly identical. Antigens 2, 5 and 6 did not show any sign of union. However, no reaction was noticed with normal rabbit and bovine sera.

### Discussions

Immuno-electrophoresis adopted in this study revealed on an average 12, 9, 11 and 10 seminal antigens in Sindhi, Jersey, Sindhi  $\times$  Jersey and Sindhi  $\times$  Friesian bulls respectively. The maximum number of seminal antigens noted was 14 in Sindhi, 10 in Jersey and 13 in cross bred bulls. Pernot (1956) demonstrated eleven antigens in guinea pig. Shulman and Bronson (1969) identified twelve antigens in human seminal plasma. Defazio and Ketchell (1972) identified 23 antigens in human seminal plasma.



Recently, Kulkarni (1985) identified 9-12 antigens in Cow and buffalo bull semen..

In the present study, three major antigens were identified to be common in all bulls irrespective of the breed. The other antigens occurred in different frequency. Some of them appeared to be specific to one breed but they were too faint for easy detection. Further, their presence or absence depends on the initial concentration of these antigens. The three major antigens were also found to be identical between breeds as confirmed by the cross reaction test.

Difference in the antigenicity of components of seminal plasma from different males have been reported by Searcy *et al* (1964) and Weil and Roberts (1965). The

hypothesis that seminal plasma of all members of the same species is antigenically identical cannot be fully supported with the results of this study. Major antigens that exhibit greatest antigenicity occurred in the seminal plasma of all bulls studied. However, there was clear indication of subtle but definite difference in seminal antigens between local, exotic and cross bred bulls.

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## Studies On The Bacterial Load In Bovine Retained Placenta.

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### ABSTRACT

Twenty-two cows with retained placenta, were treated with Steclin, Furea and Antrima boluses after manual removal of placenta. After day 21, no growth or negligible growth of bacteria was observed from the uterine fluid.

The bacterial load revealed a highly significant difference ( $P < 0.01$ ) between weeks, but not between drugs. The average pregnancy rate was 63.64 per cent for all (three) treated groups. Chi-square test was observed as non-significant between drugs with relation to fertility. However, Steclin or Furea can be used in order of preference.

\* \* \*

The bacterial etiology has not been extensively studied, though it is most important in causing damage to genital organs. Some clinical changes in retained placenta are important in practice (Kennedy, 1947) being associated with contagious abortions and other infectious diseases. The present investigation was undertaken to study the bacterial load in retained placenta cows and the effect of certain antibiotics and chemotherapeutic agents on bacterial growth and subsequent fertility of such cows.

### Materials and Methods

In the present study, the material was collected from 22 retained placenta cows presented to the Central Clinics of the College and vicinity of Bhubaneshwar. The cows were selected on the basis of normal parturition at term with retained placenta within 8-48 hours of parturition.

After recording history of each case, general clinical examination was done. The cases were then divided into three treatment groups. In all 22 cases, placenta was manually removed. The first group consisting of 7 animals was treated with Steclin (2 boluses); the second group consisting of 9 cows was treated with Furea (2 boluses) and the third group consisting of 6 cows was treated with Antrima (5 one gram) tablets. Each treatment was given only once following removal of placenta after the uterine samples were collected.

The uterine fluid was collected by using uterine catheter with all sterile precautions. A minimum of 0.5 ml uterine fluid was collected for bacteriological examination. Four such uterine samples were collected at weekly intervals, for the study of bacterial load. The bacterial load or the number of living bacteria in liquid suspension was counted adopting plate count technique as described by Malik (1967). The plates showing growth of bacteria after 24-48 hours of incubation, were placed before a colony counter and bacterial colonies counted. The calculation of bacterial number was done as under.

Total bacterial count/ml. of uterine fluid  
= Number of colonies  $\times$  dilution rate.

All the data obtained were statistically analysed as per Snedecor and Cochran (1967).

### Results and Discussion

The mean values of bacterial load (in  $10^6$  per ml. of uterine fluid) for Steclin, Furea and Antrima in 1st, 7th and 14th day were  $116.02 \pm 67.76$ ;  $6.79 \pm 4.41$  and  $0.165 \pm 0.04$ ;



355.22±229.95, 15.13±6.72 and 0.53±0.31 and 363.10±79.41, 28.70±24.81 and 0.172±0.004, respectively (Table 1). The growth on 21st day was not taken into consideration since there was nil or negligible growth. Further, it was observed that the bacterial count in cases treated with Steclin, decreased more rapidly than in cases where other two drugs were used. The overall decrease in bacterial load in all three treated groups was in close confirmation with findings of Banerjee (1966) and Kudlak and V'leck (1970).

Variations observed in all three groups might be due to the entrance of pathogenic and non-pathogenic organisms, before or at handling. Analysis of variance of the bacterial load revealed a highly significant difference ( $P<0.01$ ) between weeks, whereas no difference was marked between the drugs used in the present study (Table 1). Thus it is difficult to suggest the influence of any individual drug, though the pattern of decrease in Steclin group was in concurrence with the findings of Banerjee (1966). It can however be concluded that the variations in the bacterial count is dependant on parturient hygiene, susceptibility of individual animal and nonjudicious handling of placenta prior to treatment at the clinic, which probably favours rapid growth of organisms. Moreover, the significant difference ( $P<0.01$ ) between weekly intervals

might suggest that there is a significant effect of all three drugs with the advancement of days post-partum, which has been indicated by a negligible or non-availability of bacteria on day 21 in all groups. The non-significant difference between the drugs is suggestive of the equal and similar action of these drugs even though there was initial higher bacterial load.

The pregnancy rate was found to be highest (85.71 per cent) in cows (7) treated with Steclin, lowest (33.33 per cent) in cows (6) treated with Antrima, whereas in the Furea (9) treated group, pregnancy occurred in 66.66 per cent with an overall pregnancy rate of 63.64 per cent. However the chi-square test was non-significant between drugs. Thus though not conclusive, practically there is not much difference between drug action since effect on bacterial load did not differ significantly. Out of the drugs used, Steclin and Furea may be of choice in order of preference since the subsequent reproductive ability remains unaffected despite manual interference.

#### Acknowledgement

The authors are grateful to the Dean and Dr. P.C. Dey, Director, Central Clinics, Orissa Veterinary College for providing the facilities.

**Table 1: Total Bacterial load ( $10^6$  per ml. of uterine fluid) of retained placenta cases using different drugs at weekly intervals.**

Sr. No.	Item.	Number of cases studied	1st Day	7th Day	14th Day	21st Day	'F' Value
1.	Drugs used						
	(i) Steclin	7	116.02 ± 67.76	6.79 ± 4.41	0.165 ± 0.04	No growth	—
	(ii) Furea	9	355.22 ± 229.95	15.13 ± 6.72	0.53 ± 0.31	No growth	—
	(iii) Antrima	6	363.10 ± 79.41	28.70 ± 24.31	0.172 ± 0.004	No growth	—
2.	Between drugs	—	—	—	—	—	1.20 <sup>NS</sup>
3.	Within week intervals	—	—	—	—	—	11.76 <sup>**</sup>

Mean ± S.E. NS = Not Significant \*\* = Significant at  $P < 0.01$  level.



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## Study Of Biometry Of Buffalo (*Bos bubalis*) Ovaries.

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### ABSTRACT

A study on biometry of 128 pairs of Murrah buffalo (*Bos bubalis*) ovaries was carried out.

The length, width, thickness and weight of left ovary averaged 2.48 cms., 1.67 cms., 1.46 cms., and 3.71 gms., respectively. These figures for right ovary were 2.44 cms., 1.74 cms., 1.47 cms., and 3.77 gms. No significant difference in any of the measurements or weights between left and right ovary was observed.

\* \* \*

For the proper gynaeco-clinical assessment of each individual buffalo, it is most essential that one should be well

acquainted with normal dimensions of gonads of mature female. It is of prime importance to arrive at accurate diagnosis of normal cyclic and pathological conditions of gonads and as such it was considered necessary to undertake the present study on biometry of buffalo ovaries.

Polding and Lall (1945) studied a small number of genitalia of Murrah buffaloes. Damodaran (1958) examined 15 non-descript nulliparous buffalo genitalia. Luktuke and Rao (1962) studied 313 pairs of non-descript buffalo ovaries. Bhalla *et al* (1964) made observations on 100 non-descript adult buffalo cow genitalia. Sane *et al* (1964) studied biometry of genitalia of 1,462 Murrah buffalo cows. Sane *et al* (1965) studied



biometry of genitalia of 214 Jaffri buffalo cows. Kodagali *et al* (1971) made biometrical study on genitalia of 330 Surti buffalo cows. Kaikini (1974) studied measurements of ovaries in 966 Berari (Nagpuri) buffalo cows.

### Materials and Methods

128 pairs of clean, normal ovaries of adult Murrah buffaloes were collected from Deonar Slaughter House, Bombay. Soon after the slaughter, the material was transported to the laboratory.

Biometrical observations of ovaries were recorded as per Sane *et al* (1964). All the measurements were taken with the help of Vernier Callipers and weights using Monopan Balance for maximum accuracy. Ovarian measurements were recorded thus: (1) Length: Anterior to posterior extremity. (2) Width: From attached to free border. (3) Thickness: Diameter between the medial and the lateral surface.

### Results and Discussion

The averages of length, width, thickness and weights of 128 pairs of buffalo ovaries

along with the coefficients of variations are presented (Table 1). All the means of measurements and weights, of left and right ovary were compared applying the Student's 't' test. The 't' values were non-significant indicating that there was no significant difference in any of the measurements or weights between the left and right ovary.

The average length of left and right ovary, in the present study was 2.48 and 2.44 cms., respectively which is in accordance with the observations of Damodaran (1958), Luktuke and Rao (1962) and Bhalla *et al* (1964). The average width of left and right ovary was 1.67 and 1.74 cms., respectively which are close to the observations of Damodaran (1958). The average thickness of the left and right ovary was 1.46 and 1.47 cms., respectively which is in accordance with the observations of Luktuke and Rao (1962) and Bhalla *et al* (1964). The average weight of the left and right ovary was 3.71 and 3.77 gms., respectively which is in accordance with the findings of Sane *et al* (1965).

Table-1 Biometry of Buffalo Ovaries

Ovary	No. of observations	Mean $\pm$ S.E.	C.V. Percent
<b>LEFT</b>			
Length (cm)	128	2.48 $\pm$ 0.03	15.04
Width (cm)	128	1.67 $\pm$ 0.03	20.63
Thickness (cm)	128	1.46 $\pm$ 0.03	19.61
Weight (gm)	128	3.71 $\pm$ 0.08	42.77
<b>RIGHT</b>			
Length (cm)	128	2.44 $\pm$ 0.04	18.23
Width (cm)	128	1.74 $\pm$ 0.04	23.33
Thickness (cm)	128	1.47 $\pm$ 0.02	18.85
Weight (gm)	128	3.77 $\pm$ 0.08	40.46



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## Synchronization Of Oestrus In Post-Partum Anoestrous Buffaloes (*Bubalus bubalis*) With Short-Term Steroid Treatment

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### ABSTRACT

Thirty post-partum anoestrous buffaloes were divided into three groups of 10 animals each, Group A, B and C. Group A received 25 mg progesterone daily for five days 1/M; Group B received same treatment as Group A, in addition to injection of 5 mg estradiol valerate on 6th day and Group C served as control. The number of animals in synchronized oestrus were 3, 9 and 2; the number of days from last injection to occurrence of oestrus were  $4.33 \pm 0.71$ ;  $3.33 \pm 0.22$  and  $8.5 \pm 0.35$  for groups A, B and C respectively. The number of animals conceiving in induced oestrus were 1, 4 and 0 for the three groups respectively.

\* \* \*

Various progestational preparations have been used singly or in combination with oestrogens as intravaginal pessaries or device

for synchronization of oestrus in anoestrous buffaloes for a period of 9 to 12 days (Rao and Rao, 1977). Although, there are some reports in cows regarding induction of oestrus with short-term injectable steroid treatment (Gonzalez and Ruiz, 1978; Gonzalez *et al.*, 1980), there is paucity of information in buffaloes employing such a regime. Therefore, the present experiment was undertaken to evaluate efficacy of short-term steroid treatment for induction and synchronization of oestrus in post-partum anoestrous buffaloes.

### Materials and Methods

The experiment was conducted on 30 post-partum (100-200 days) buffaloes belonging to Livestock Farm, Adhartal, J.N.K.V.V., Jabalpur (M.P.) during January to April, 1983. Animals showing true anoestrus were



randomly divided into three groups of ten each.

Group A: received intramuscular injection of 25 mg progesterone (Proluton depot, German Remedies Pvt. Ltd., Bombay) for a period of 5 days.

Group B: received the same treatment as Group A and in addition estradiol valerate 5 mg on 6th day (Progynon depot, German Remedies Pvt. Ltd., Bombay).

Group C: served as control.

All the animals were closely observed for the signs of oestrus. Those detected in induced oestrus were mated and pregnancy diagnosis was done 45th day after service. Ovulation was confirmed by gynaeco-clinical finding of corpus luteum 10th day after induced oestrus.

### Results and Discussion

The results show maximum response of Group B indicating superiority of that treatment (Table I). A high degree of oestrus synchronization and acceptable fertility has been reported following the use of PRID or similar progesterone releasing devices during breeding season in buffaloes (Rao and Rao, 1977; Rao and Rao, 1983; Singh *et al.*, 1984). The results obtained in this experiment corroborate with these findings. However, a higher percentage of animals responded to

this short-term steroid treatment than that reported with oral feeding of MGA or MGA+ estradiol benzoate for a period of 14 days (Agarwal *et al.*, 1985). This may possibly be due to different preparations of the steroids employed, and the period for which the drugs were administered.

The response to the treatment is better than earlier reports in cows with same injection schedule (Gonzalez and Ruiz, 1978; Gonzalez *et al.*, 1980). These workers tried the treatment in 30 days post-partum cows. It is quite likely that response obtained to the present experiment in buffaloes was better due to difference in post-partum period (100-200 days).

Thus, it appears that the short-term steroid treatment used in this experiment is effective in inducing oestrus in post-partum buffaloes. However, it should be tried on large number of animals to evaluate its effect on conception rate.

### Acknowledgements

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Table 1: Response to various treatments

Sr. No.	Treatment Group	Interval from last injection to oestrus	No. of animals responding/Total no. of animals(n)	No. of animals ovulated	No. of animals conceived
1	A	4.33 $\pm$ 0.71	3/10	2	1
2	B	3.33 $\pm$ 0.22	9/10	6	4
3.	C	8.5 $\pm$ 0.35	2/10	2	0



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## Heterospermic Insemination And Fertility In Mehsana Buffaloes\*

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Heterospermic semen has been reported to enhance progressive sperm motility and conception rates as compared to homospermic semen in cattle (Veselov, 1966; Nelson *et al*, 1975), sheep (Anon, 1985) and goats (Sinha *et al*, 1983). Similar studies on mixing of semen of 2-3 bulls and its effect on progressive motility of spermatozoa have been carried out with no beneficial results in different buffalo breeds (Mukharjee and Rajwar, 1970; Narayan and Singh, 1971). However, reports on field trials of heterospermic inseminations in buffaloes and their fertility results are few (Singh *et al*, 1971; Jani *et al*, 1986). Hence a study on heterospermic inseminations with frozen semen was undertaken towards increasing fertility and the results have been reported.

### Materials and Methods

The semen from 6 Mehasana buffalo bulls was obtained at weekly intervals in 2-3 ejaculates per collection schedule, using artificial vagina. Immediately after collection, semen was evaluated for the various seminal attributes (Tomar, 1970). Semen samples with optimum quality (minimum + 3 mass activity and 70% motility) were diluted and processed further in tris fructose yolk glycerol diluent (FAO, 1979) keeping 25-30 million sperms/0.5 ml straw. Semen from 4 groups of two bulls each (MH-1 + MH-8; MH-3 + MH-8; MH-2 + MH-4 and MH-2 + MH-5) was utilized to produce heterospermic semen by mixing equal volume of diluted semen from each bull and the remaining quantity served as homospermic controls. The straws were

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frozen on liquid nitrogen vapour after 5 hours of equilibration at 5°C and adopting thermocole freezing unit (Jani *et al*, 1986). Frozen semen doses so produced were stored for 15 days before use. Thawing was effected by immersing the straws in water bath at 38°C for 30 seconds. Fertility trials, using 1726 heterospermic and 5264 homospermic frozen semen doses, were conducted during high breeding season (Sept-Feb) in Mehsana buffaloes under field conditions of ICDP, Mehsana. Well trained livestock inspectors were involved in the inseminations, follow-up and pregnancy diagnosis work, which was confirmed 90 days later. The data were analysed statistically using chi-square test (Snedecor and Cochran, 1971).

### Results and Discussion

The results of field fertility trials using 4 groups of two bulls shows that the overall mean conception rate obtained with 1,221 and 4,324 followed heterospermic and homospermic inseminations was 49.05 and 45.47 percent, respectively (Table I). The difference was significant ( $P < 0.05$ ). Among the 4 groups of bulls presently tested, only group-I bulls gave significantly higher conception rate with heterospermic semen (50.49%) as compared to homospermic inseminations from the same two bulls (44.92%). With the other three groups, the

differences were statistically nonsignificant. Apparently higher conception rates were however, obtained with heterospermic inseminations in group-II and IV as compared to those of homospermic inseminations of these bulls (Table I). The results obtained under the present study are in close agreement with those reported by Singh *et al* (1971) and Jani *et al* (1986) in buffaloes.

The nonsignificant differences obtained in the spermatozoal motility at pre and post freeze between homospermic and heterospermic semen under report are in close agreement with the findings of Mukharjee and Rajwar (1970) and Narayan and Singh (1971) in Murrah buffalo bulls.

It was concluded that the heterospermic inseminations help to enhance fertility rate when semen from suitable buffalo bulls is mixed.

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**Table—1 Comparison between conception rates of heterospermic and homospermic frozen semen inseminations in buffaloes.**

Group	Buffalo bulls	Heterospermic AI			Homospermic AI			X <sup>2</sup> value (1 df)
		No. of fresh AI	No. Conceived.	C.R. %	No. of fresh AI	No. Conceived	C.R. %	
I	MH-2 + MH-5	401	204	50.49	1832	823	44.92	4.687*
II	MH-2 + MH-4	140	65	46.42	825	387	46.90	0.111 <sup>NS</sup>
III	MH-1 + MH-8	337	158	46.80	502	208	41.43	2.425 <sup>NS</sup>
IV	MH-3 + MH-8	343	172	50.10	1165	548	47.03	1.025 <sup>NS</sup>

\* = significant at 5% level; NS = nonsignificant.



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## Placental Expulsion Time And Serum Progesterone In Surti Buffaloes.

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### ABSTRACT

Serum progesterone estimated by radio Immuno Assay (RIA) technique during early post-partum has been correlated with placental expulsion time in Surti buffaloes. Three groups of animals based on their placental expulsion time were studied. Group I Placental expulsion in less than 4 hours; Group II within 6 to 8 hours and Group III more than 10 hours. Overall progesterone concentration for Group I, II and III was  $0.34 \pm 0.04$ ;  $0.86 \pm 0.12$  and  $0.62 \pm 0.09$  ng/ml respectively.

Progesterone status during early post-partum does affect the placental expulsion time, may be through disturbing the ratio of estrogen to progesterone.

\* \* \*

Expulsion of placenta within normal time is an essential factor of normal reproductive efficiency of post-partum dairy animals. Average time recorded for Surti buffaloes is 5 hours 6 minutes (Anon, 1983). Many workers have worked on the levels of serum progesterone in relation to retention of placenta (Dutta and Dugwekar, 1984, 1986; Rajpal and Vadnere, 1985). However, there is no literature available for chronological progesterone levels in relation to placental expulsion time. Hence an attempt was made to correlate the progesterone levels during early post-partum stages with the expulsion of placenta in Surti breed of buffaloes.

### Materials and Methods

Fifteen pluriparous Surti buffaloes of Reproductive Biology Research Unit Farm



were studied. All the animals were normal, clinically healthy and had calved. They were milked within 30 minutes of foetal expulsion. The calves were weaned right from birth. The buffaloes were divided in three groups of five each, on the basis of the time taken for expulsion of placenta. Group I: Placental expulsion within 4 hours. Group II: Placental expulsion between 6 to 8 hours. Group III: Placental expulsion after 10 hours.

Blood was collected from jugular vein at hourly intervals starting immediately after expulsion of calf (0 hours) and continued till complete expulsion of placenta. The last two collections were taken immediately after expulsion of complete placenta (PE-I) and one hour thereafter (PE-II). Serum was separated and stored at  $-20^{\circ}\text{C}$  till analysed.

Serum progesterone was estimated by RIA technique (Kubasik, 1984) with some modifications. Labelled antigen (with  $1^{25}$ ), antibody coated tubes and standards were procured from Los Angeles (U.S.A.). Coated tubes after adding samples and tracer were incubated for 3 hours at room temperature. Thereafter, the material was decanted carefully on absorbing rack. These tubes were read in Gamma Counter. The standard progesterone curve was linear in the range of 0.1 to 40 ng/ml. All the samples were run in duplicate. The sensitivity of the assay was to detect the limit of approximately 0.05 ng/ml. Intra assay variation calculated on high, low and medium samples was 7.2%, while inter assay variation was 5.9%. Cross reactivity of the antibody with progesterone,  $20\alpha$  dehydroprogesterone and 17-hydroxyprogesterone was 100%, 20% and 0.3% respectively.

The data was statistically analysed using factorial design (Steel and Torrie, 1960).

### Results

Average progesterone concentration for Group I, II and III was  $0.34\pm0.04$ ;  $0.86\pm0.12$

and  $0.62\pm0.09$  ng/ml respectively (Fig. 1). Progesterone concentration at 0 hours for group-I was remarkably low ( $0.40\pm0.09$  ng/ml) compared to that of group-II ( $1.62\pm0.32$  ng/ml) and group-III ( $0.68\pm0.11$  ng/ml) (Table 1). Progesterone concentration for group-I showed very steady fall throughout the study and the levels fluctuated within very narrow range of 0.22 to 0.41 ng/ml without any significant variation. The progesterone concentration in group II and III immediately after parturition (0 hours) was remarkably high and variation for stages were significant ( $P<0.01$ ). There was significant increase in the level at 3 hours post-partum in both the groups but in group-III, the rise re-appeared again at 7 hours post-partum (Fig. 1). Thereafter the level decreased steadily in both the groups till the complete expulsion of placenta.

### Discussion

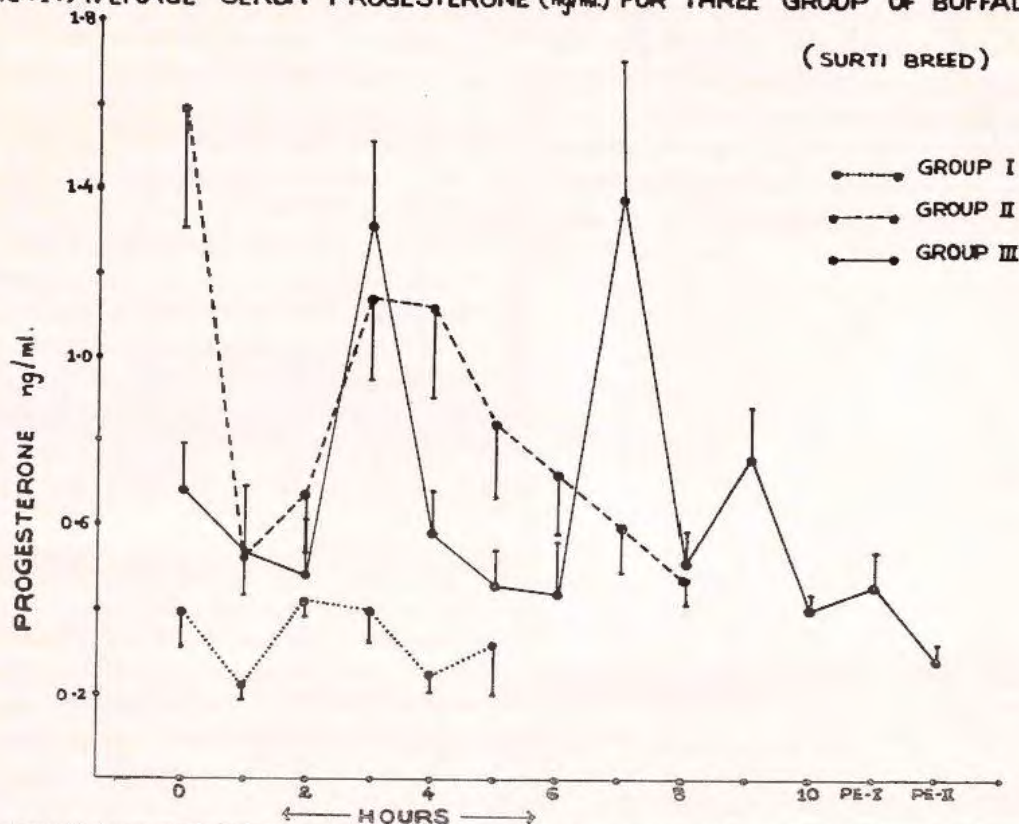
The overall average levels observed in this study are comparable with that of Dutta and Dugwekar (1984) and Rajpal and Vadnere (1985) in riverine buffaloes; Parera (1979), Kamonpatana *et al* (1981) and Jainudeen *et al* (1981) in Swamp buffalo and Inaba *et al* (1980) and Dutta and Dugwekar (1986) in cows.

Results obtained by Chow *et al* (1977); Dutta and Dugwekar (1986) for Cows and Dutta and Dugwekar (1984), Rajpal and Vadnere (1985) for cases of retained placenta in buffaloes showed that the level of progesterone after calving was significantly high. In the present studies, animals of Group II and III with delay in placental expulsion also showed remarkably higher level of progesterone immediately after parturition (Fig. 1 and Table 1). This reveals that serum progesterone concentration immediately after foetal expulsion influences placental expulsion with higher levels delaying expulsion of placenta.



FIG : 1 : AVERAGE SERUM PROGESTERONE (ng/ml.) FOR THREE GROUP OF BUFFALOES :

(Surti Breed)



Table—1 Average progesterone concentration (ng/ml) for 6 stages of three groups (Mean  $\pm$  SE)

Stages	Post-partum Hours					
	0	1	2	3	PE-I	PE-II
Group-I	0.40 $\pm$ 0.09	0.22 0.04	0.43 0.04	0.41 0.06	0.25 0.04	0.33 0.13
Group-II	1.62 $\pm$ 0.32	0.52 0.09	0.67 0.11	1.13 0.18	0.61 0.11	0.47 0.06
Group-III	0.68 $\pm$ 0.11	0.54 0.15	0.48 0.12	1.32 0.19	0.47 0.07	0.29 0.02
$\bar{x}$	0.90	0.43	0.53	0.95	0.44	0.36
S.E.	0.37	0.10	0.073	0.28	0.10	0.054

0 hrs. — Immediately after calving; PE-I — Immediately after complete expulsion of placenta;  
PE-II — One hour after placental expulsion.



Animals of Group II which had placental expulsion within 8 hours showed increase in progesterone concentration around 3 hours post-partum. Likewise the animals of group II which took more than 10 hours for placental expulsion showed such increase twice; at 3 hours and 7 hours post-partum stage (Fig. 1). Actual source for progesterone immediately after calving is not known but may be from follicular tissue (Rowling *et al.* 1980; Webb *et al.* 1980) or its leaching from deposits in fatty tissue (Hopman *et al.* 1979). This

indicates that increase in progesterone concentration during early post partum stages definitely affects the placental expulsion possibly by disturbing its ratio, with estrogen.

It is concluded that there is a close association between serum progesterone concentration during early post-partum stages and placental expulsion. Thus, serum progesterone concentration during early stages may be the deciding factor for placental expulsion.

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## Histochemistry Of Buffalo Uterus Affected With Uterine Torsion

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### ABSTRACT

Results showed PAS positive reaction in the basement membrane of uterine and vascular epithelium, infiltrating cells of lamina propria, glandular epithelium, smooth muscle cells of myometrium and mesothelial cells of epimetrium. Bound lipids were abundant in the basement membrane in the inflammatory cells of lamina propria and in the blood vascular epithelium.

\* \* \*

Uterine torsion is a major obstetrical condition of pregnant buffalo. Histochemical studies of the affected uterus have not been studied so far, but histochemistry can be a useful tool alongwith histopathology and gross pathology, to determine its impact on subsequent fertility of the affected animals. Histochemical studies of uterine torsion were carried out and are reported.

### Material and Methods

A small piece of inter-caruncular tissue was collected from 12 buffaloes affected with uterine torsion, during caesarean section. After collection, the tissues were directly transferred into 10% neutral buffered formalin. These tissue samples were then processed for histochemistry as per the procedures described by Luna (1968).

### Results and Discussion

Results and histochemical studies have been presented in Table I. Endometrial surface epithelium was generally absent but was observed in a small patch in one case. It

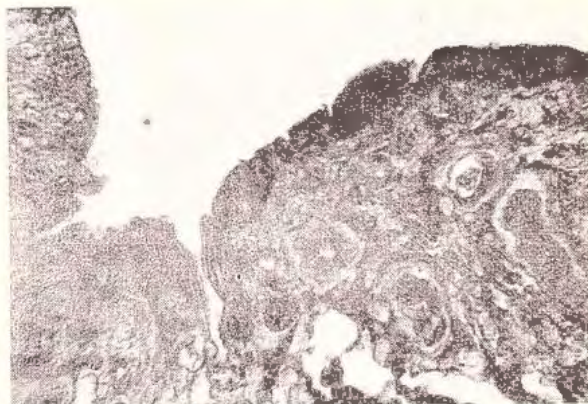


Fig. 1: Photomicrograph showing moderate reaction for bound lipids in the surface lining of endometrium. Sudan Black x 400.

was mildly PAS reactive and sudan black positive. The bound lipid particles were observed in a mild coarse granular fashion in supra and infranuclear zones of the cells (Fig. 1). The basement membrane was also moderately PAS reactive. The reaction for the bound lipid was variable ranging from inconsistent or mild to intense. The lining epithelium of the remnants of glands showed moderate to strong reaction with PAS stain and mild to moderate consistency of aldehyde fuchsin revealing concentration of different types of carbohydrates in the cells. The reaction for bound lipid was mild to moderate in a fine granular form. The intra-luminal secretion of the gland was intensely reactive to PAS stain indicating rich mucoproteinous

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complex but lack of aldehyde fuchsin reaction. The reaction for lipid was moderate. Thus, it appears that the secretion is mucoproteolipid in composition and not a free carbohydrate.

The lamina propria which was heavily infiltrated with cellular material in some cases was strongly PAS reactive and also mildly to strong sudan black reactive. Reaction for aldehyde fuchsin was inconclusive. The placental remnants and migratory trophoblasts were inert to aldehyde fuchsin, but mildly to strongly reactive for PAS and mildly to moderately for bound lipids in sudan black stain. The endothelium of the blood vessels and capillaries also showed sudan black and PAS staining for bound lipids and carbohydrates in moderate intensity (Fig. 2).

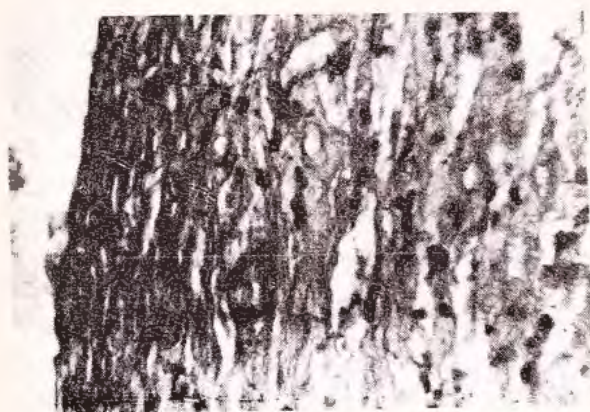


Fig. 2: Photomicrograph showing PAS positive infiltrating cells, endothelium of capillaries, basement membrane and sub-epithelial propria. PAS x 400.

**Myometrium:** The myometrium was mostly degenerated and exhausted as indicated by very mild to moderate PAS staining of smooth muscles and light to sudan black (particularly along their cell membrane) which normally should have been rich in these features.

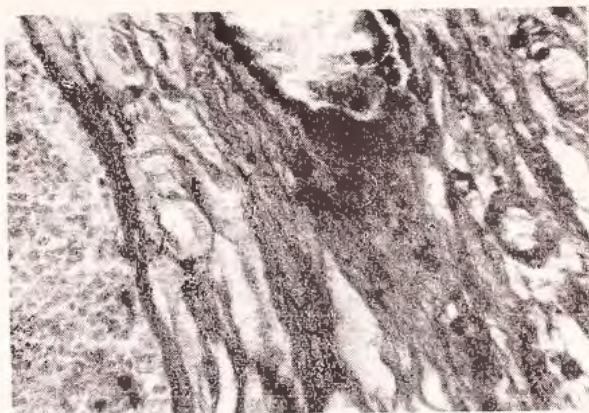


Fig. 3: Photomicrograph showing PAS positive reaction in intravascular and inflammatory polymorphs and the endothelium of blood vessels. PAS x 400.

The glycogen concentration at the implantation site particularly in the muscular layer has been found to be always high throughout the gestation period in hamsters (Chugh, 1978), which is stated to be important to meet the changed activity of the musculature during this phase of reproduction. The histochemical reactions were more evident in the ectoplasm than in the endoplasm. On the contrary, in the stratum vasculare, the blood vessels revealed moderate to stronger concentration of the carbohydrate and bound lipids (Fig. 3). Vascular wall was mildly to strongly reactive for bound lipids and moderate to strong for glycoproteins. Reaction for aldehyde fuchsin reactive carbohydrates normally encountered in the vascular elastin became homogeneously sprayed and sometimes indistinct due to elastolysis.

**Epimetrium:** Connective tissue membrane was negative for lipids and carbohydrates but was positive for elastin as revealed by aldehyde fuchsin staining. On the contrary, muscle cells did not show aldehyde fuchsin, but showed slight affinity to PAS and sudan black. The lining mesothelium was mild to moderate reactive for both lipids and



carbohydrates. The epimetrium has also been reported to be relatively histochemically poorly reactive in normal cycling buffalo uteri (Harcharan Singh, 1983).

It can be concluded that PAS and sudan black for bound lipids were not sufficient to give any diagnostic or prognostic clues about the disease.

**Table 1: Histochemical Reaction of the Uterus after Torsion.**

Sr. No.	Tissue	SBB	PAS	AF
1.	<b>Endometrium</b>			
	Basement membrane	± to ++	+	—
	Lamina propria	± to ++	++	±
	Trophoblast	± to +	± to ++	—
	Glandular epithelium	± to +	+ to ++	± to +
	Glandular (lumen) contents	+	++	—
	Capillary endothelium	+	+	—
2.	<b>Myometrium</b>			
	Muscle	±	± to +	?
	Blood vessels	± to ++	± to ++	±
3.	<b>Epimetrium</b>			
	Muscle	± to +	±	—
	Connective tissue	—	—	±
	Mesothelium	± to +	± to ++	± to +
<hr/>				
SBB —	Sudan Black B	± — Inconsistent or V/VV mild		— Absent
PAS —	Periodic Acid Schiff	+ — Moderate		++ - Intense
AF —	Aldehyde Fuchsin	? — Non specific reaction		+++ — Very strong

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## Effect Of 'Hormotone' Therapy On Involution Of Cervix And Vulva In Cows And Buffaloes.

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### ABSTRACT

The effect of 'Hormotone' liquid therapy on the involution of cervix and vulva of 13 cows and 18 buffaloes was studied. The average time required for involution of cervix of cows was  $18.92 \pm 1.11$  and  $19.63 \pm 1.35$ , vulva  $10.46 \pm 1.11$  and  $9.63 \pm 1.57$  days in experimental and control groups whereas in buffaloes the corresponding values were: Cervix  $19.77 \pm 0.51$ ,  $20.67 \pm 1.11$  and vulva  $12.22 \pm 1.11$ ,  $13.17 \pm 2.06$  days. The difference was non-significant in both the groups.

\* \* \*

The rate of post-partum fertility primarily depends on the time required for involution of uterus and recurrence of first fertile estrus. More rapid the involution of uterus, the earlier will be occurrence of estrus. Earlier studies on involution of uterus in cows revealed that the efficacy of 'Hormotone' was highly significant in reducing the involution period of uterus in cows, whereas in buffaloes, it had no significant effect (Dindorkar *et al*, 1982). Information on the involution of cervix and vulva is variable and scanty. Hence, studies on the effect of 'Hormotone' Therapy on involution of cervix and vulva in cows and buffaloes are placed on record.

### Material and Methods

21 cows and 24 buffaloes of the Livestock Instructional Farm, Akola were selected for

the present studies after a thorough gynaecological examination. On this farm, regular detection of cows buffaloes in heat is practiced by parading teaser bull in the cow-byres, followed by selective natural breeding. The experimental animals were healthy and free from any genital infections and kept under identical conditions of care and management.

The experimental groups comprised of 13 (8 cross-bred + 5 Sahiwal) cows and 18 buffaloes, while control group consisted of 8 (6 cross-bred + 2 Sahiwal) cows and 6 buffaloes. Immediately after parturition, within half an hour, 'Hormotone' liquid 200 ml. (Charak Pharmaceuticals, Bombay) was drenched to each experimental animal. On the second and third day after parturition 100 ml of 'Hormotone' was administered once daily.

All the ingredients of 'Hormotone' are uterine stimulants and are capable of increasing ovulatory functions. It is also recommended by the firm for efficient use in expulsion of placenta, involution of uterus and onset of early post-partum estrus in animals.

After parturition, the gynaeco-clinical examination of all experimental and control animals was carried out daily in the morning after milking. Efforts were made to confirm the exact time when cervix and vulva

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completely involuted, without further reduction in length, size and shape, and when four to five examinations at 24 hourly intervals showed no further reduction in size and length of cervix and vulva, the initial date of observation was taken as the date of complete involution.

### Results and Discussion

#### *Involution of Cervix:*

i) *Cows:* The average time interval required for involution of cervix in experimental and control group of cows was  $18.92 \pm 1.11$  days (range 13.0 to 26.0 days) and  $19.63 \pm 1.35$  days (range 13.0 to 24.0 days) respectively. The average diameter of involuted cervix in experimental and control group of cows was  $4.61 \pm 0.26$  and  $4.89 \pm 0.51$  cms. respectively. The difference was non-significant (Table-1).

These findings are in partial agreement with Zemjanis (1962), who reported an average interval of 3 weeks, but in the present study the involution of cervix was faster than that of uterine cornua (21.38 days). However, the present findings are much lower than those of Morrow *et al* (1969), who reported an average interval of 30 days in normal calvings. Difference might be due to breed, environment and managerial variations.

ii) *Buffaloes:* The average time required for the involution of cervix in experimental and control group of buffaloes, was  $19.77 \pm 0.51$  (range 17.0 to 24.0) days and  $20.67 \pm 1.11$  (range 16.0 to 22.0) days respectively. The difference was non-significant (Table 1).

The average diameter of involuted cervix in experimental and control group of buffaloes was  $3.76 \pm 0.14$  cm and  $3.59 \pm 0.22$  cm respectively (Table 1).

The present findings are in agreement with that of Zemjanis (1962), who reported 3 weeks time for complete involution of cervix in cows. However, the present findings are much lower than those of Chauhan *et al* (1976), who reported an average time of  $44.0 \pm 2.14$  days. The difference might be due to breed, environmental and managerial variations.

#### *Involution of Vulva:*

i) *Cows:* The involution of Vulva in experimental and control group of cows averaged  $10.46 \pm 1.11$  (range 5.0 to 21.0) days and  $12.50 \pm 1.75$  (range 4.0 to 18.0) days respectively. The difference was non-significant (Table 2).

The present findings are much lower than those reported by Zemjanis (1962) as 2 weeks after parturition in normally calved animals, whereas the findings of Dhirendranath and Mishra (1978) as  $20.63 \pm 0.30$  days are much higher than the present findings. The difference might be due to breed, environment and managerial variations.

ii) *Buffaloes:* The involution of vulva in experimental and control group of buffaloes averaged  $12.22 \pm 1.11$  (range 4 to 20) days and  $13.17 \pm 1.06$  (range 9 to 21) days respectively. The difference was non-significant (Table 2).

The present findings are not in agreement with the findings of Bhalla *et al* (1966), who reported an average interval of  $23.6 \pm 1.01$  days in 45 buffaloes. The difference might be due to breed, environment and managerial variations.

### Acknowledgements

The free gift of 'Hormotone' liquid from Charak Pharmaceuticals, Bombay is duly acknowledged.



**Table 1: Analysis of Variance for involution of cervix.**

Sr. No.	Parameter	Group	Mean	S.E.	S.D.	't' value	Significance.
<b>Cows</b>							
1.	Time interval	Control	19.63	1.35	3.81	0.4084	N.S.
		Experimental	18.92	1.11	3.99		
		Control	20.67	1.11	2.71	0.2998	N.S.
		Experimental	19.77	0.51	2.61		
<b>Bufs</b>							
2.	Diameter of involuted Cervix	Control	4.89	0.50	1.42	0.5450	N.S.
		Experimental	4.61	0.26	0.94		
		Control	3.59	0.22	0.52	0.5701	N.S.
		Experimental	3.76	0.14	0.66		

**Table 2: Analysis of time required for involution of vulva.**

Sr. No.	Species	Group	Mean	S.E.	S.D.	't' value	Significance.
1.	Cows	Control	12.50	1.75	4.44	0.5365	N.S.
		Experimental	10.46	1.11	4.00		
2.	Buffaloes	Control	13.17	2.06	5.04	0.4222	N.S.
		Experimental	12.22	1.11	4.70		

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## Postnatal Development Of Testis And Epididymis Of Deccani Ram\*

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### ABSTRACT

Studies on the postnatal growth of testis in Deccani ram lambs showed that the mean testicular weight increased from 6.95 gm at 120 days to 110.35 gm at 240 days of age.

The external and Luminal diameters of the seminiferous tubule were  $114.84 \pm 4.95 \mu\text{m}$  and  $67.71 \pm 3.94 \mu\text{m}$  respectively at 120 days of age which increased to  $180.18 \pm 5.9 \mu\text{m}$  and  $54.05 \pm 3.76 \mu\text{m}$  respectively at 240 days of age.

At 120 days of age the right testis consisted of only sex cords while the left testis was luminated. At 150 days the right testis was luminated and was lined by spermatogonia. At 180 days, the seminiferous tubules of both the testes had spermatogonia, primary and secondary spermatocytes and spermatids. At 210 days of age, all the stages of spermatogenesis were noticed and at 240 days of age all the stages of spermatogenesis along with active primary spermatocytes were noticed. It is concluded that at 240 days of age the Deccani rams are sexually mature and can be used for breeding purpose.

\* \* \*

Information on the changes that occur in the postnatal development of testis and epididymis in domestic animals is very valuable to determine the onset of puberty and maturity in breeding stock. But such information is meagre in Indian breeds of sheep. Hence, the present investigation was taken up to study the structural changes that occur in testis and epididymis during 120 to

240 days of postnatal development in Deccani ram with a view to determine suitable age for breeding purpose.

### Material and Methods

Ten Deccani Ram lambs obtained from Government Livestock farm were used in this study. They were maintained on a daily ration consisting of 200-250 gm of premixed feed, besides 2.5-3.0 kg of greens per lamb. The testis and epididymis were collected immediately after slaughter from lambs sacrificed at 120, 150, 180, 210 and 240 days of age.

The body weight and testicular weight of each animal at slaughter were recorded. The length, breadth and thickness of testis were measured with the help of vernier callipers. The testicular tissue pieces obtained from apical, middle and caudal part of the testis, and head, body and tail of the epididymis were preserved in Bouin's fluid for 24 hours. The tissue pieces were dehydrated, cleared and embedded in paraffin. Paraffin sections 5-6  $\mu\text{m}$  thick were stained with Haematoxylin Eosin for routine study and PAS reaction for Carbohydrates (Singh and Sulochana, 1978).

Twenty five seminiferous tubules selected at random from each section were selected to measure the diameter of the seminiferous tubules. The external diameter (from basement to basement membrane) and luminal diameter (from the luminal margin of the basal cells to luminal margin at the

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opposing basal cells) of seminiferous tubules were measured with the help of oculometer.

## Results

The shape of the testis in all the animals studied, was oval to ovoid. The body weights at 120, 150, 180, 210 and 240 days of age were 12.5, 14.0, 15.75, 18.10 and 20.25 kg respectively. The testicular weights were found to be 6.95, 40.22, 42.02, 62.25 and 110.35 gm respectively for the same period. The mean testicular weight of the left testis was found to be heavier than the right testis in all the age groups.

*Seminiferous Tubules:* The data on the diameter of the seminiferous tubules at different ages/body weights are given in Table 1. The growth of the seminiferous tubules appeared to be sigmoid type.

*120 Days:* At 120 days of age, the testicular parenchyma of right testis consisted of sex cords only. The epithelial lining of these sex cords was mainly composed of small gonocytes, resting on PAS positive basement membrane. The small gonocytes had basophilic cytoplasm and darkly stained oval nuclei. The large acidophilic gonocytes were seen in the centre of the cords (Plate 1).

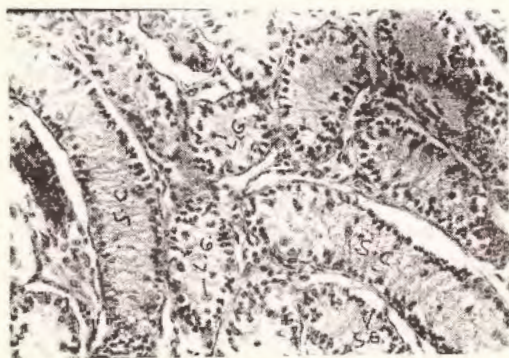


Plate 1: Photomicrograph from 120 days right testis showing sex cords (S.C.), Small gonocytes (S.G.) and Large gonocytes (L.G.).

However, in the left testis, immature seminiferous tubules were recorded with a distinct lumen. Most of the seminiferous tubules were lined by spermatogonia and primary spermatocytes. Degenerating large gonocytes were also observed (Plate 2). The epididymal duct was lined by pseudo-stratified ciliated columnar epithelium.

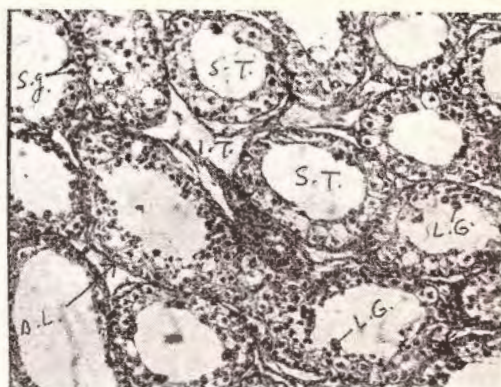


Plate 2: Photomicrograph from 120 days left testis showing luminated seminiferous tubule (S.T.) with prominent basal lamina (B.L.) interstitial tissue (I.T.), Large gonocytes (L.G.) and Spermatogonia (Sg).

*150 Days:* In the right testis, most of the sex cords were seen in the process of changing from straight to convoluted seminiferous tubules with well defined lumen. The germinal epithelium consisted of spermatogonia and one layer of primary spermatocytes. In the left testis, some seminiferous tubules were having two layers of primary spermatocytes, mostly in zygotene stage. The lumen of the epididymis was often found empty.

*180 Days:* In both the testes, most of the seminiferous tubules were having all cell types of germinal epithelium like spermatogonia, primary and secondary spermatocytes and spermatids. The spermatids were observed in two forms- immature and mature forms. In some seminiferous tubules, free spermatozoa



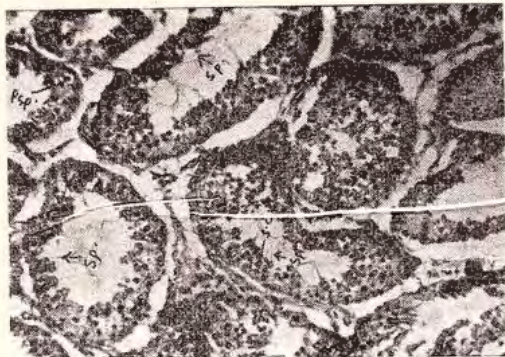


Plate 3: Photomicrograph from 180 days testis showing primary spermatocytes (P.S.P.), Spermatids (Spt.) and free spermatozoa (Sp.) in the lumen.

were seen in the lumen (Plate 3). Blind receptacle between the tubuli recti and rete-testis were recorded signifying the sexual immaturity.

**210 Days:** All the stages of spermatogenesis were seen. The spermatogenic wave had set in and in many seminiferous tubules spermatozoa were attached to sertoli cells (Plate 4). Basement

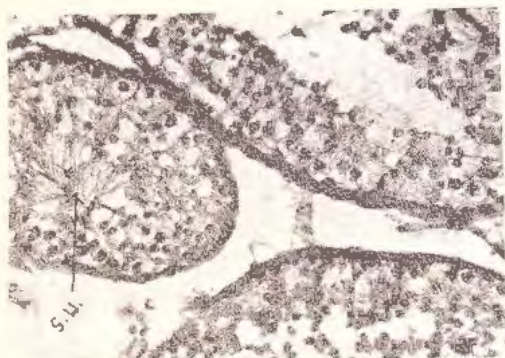


Plate 4: Photomicrograph from 210 days testis showing all stages of Spermatogonia. The spermatogenic wave (S.W.) was set in.

membrane, acrosomal cap and body of spermatozoa showed intense PAS-positive reaction. The lumen of the epididymis contained clumps of spermatozoa.

**240 Days:** The seminiferous tubules were lined by all cell types of germinal epithelium. The primary spermatocytes were active and mostly were in zygotene stage (Plate 5). The interstitial space contained fully mature Leydig Cells. The epididymis was full with clumps of spermatozoa and Whirl-like waves were noticed (Plate 6).

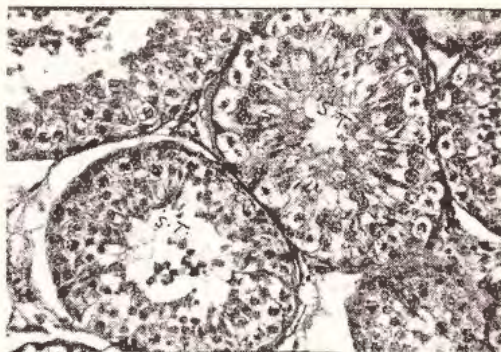


Plate 5: Photomicrograph from 240 days testis showing the cross section of active seminiferous tubule (S.T.).

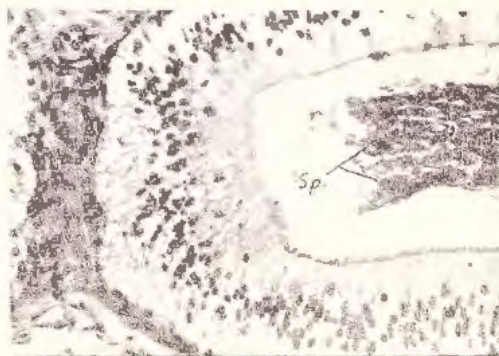


Plate 6: Photomicrograph from 240 days epididymis showing the lumen filled with clumps of Spermatozoa (Sp.).

## Discussion

In the present study, the growth pattern of the seminiferous tubule was recorded as sigmoid in nature. Similar growth pattern was reported by Skinner *et al* (1968). The size of the seminiferous tubules was found to be significantly correlated with age, body weight



and testicular weight in Deccani ram. This is in keeping with the observations reported by Carmon and Green (1952) in rams and Yao and Eaton (1954) in goats.

The lumination of the sex cord was noticed at 120 days of age and the process of lumen formation was completed at 180 days of age in the present study. No data in sheep is available for comparison. However, Kesava Reddy (1983) reported initiation of lumen formation at 120 days and completion at 210 days of age in goats. The sex cords were lined with two types of cells- the small and large gonocytes. The small gonocytes are the precursors of the spermatogonia and sertoli cells. The large gonocytes which were centrally located degenerated subsequently. Courot (1962) and Spasford (1962) mentioned that sertoli cells proliferated at 120 days of age in rams. This is in accordance with the present findings. However, Skinner *et al* (1968) recorded the differentiation of sertoli cells at 70 days of age in Suffolk rams.

The first appearance of spermatogonia and primary spermatocytes was observed at 120 days of age in the present study, whereas Skinner (1970) reported the same at 112 days of age in Namaqua and Pedi rams. The early appearance of the primary spermatocytes observed at 63 days in South Down ram (Philips and Andrews, 1936) and 56 days in

Hampshire rams (Carmon and Green, 1952) may be due to differences in breed and growth rate. The secondary spermatocytes were noticed at 150 days and spermatids at 180 days in the present study. In few seminiferous tubules, spermatozoa were also seen at 180 days of age.

On the other hand, Carmon and Green (1952) reported the appearance of these cells at an earlier age in exotic rams which might be due to genetic make up and fast growth rate attained in these animals. It was also observed in this study that the receptacles were not opened at 180 days of age, as the spermatozoa were not formed. Similar observations were made by Singh and Bhardwaj (1980) in Camel. In the present study, mature interstitial cells appeared at 240 days of age and very few spermatozoa could be seen in epididymal lumen at 180 days, though the lumen was full of spermatozoa at 240 days of age. But in Merino rams, sperms were observed between 125 and 154 days (Dunn, 1955). The appearance of Germ cells in Deccani Ram was found to be at a comparatively later age than that reported in exotic rams.

The age of puberty in the present study can be taken as 240 days when all the stages of spermatogenesis were observed. It is concluded that the Deccani rams can be used for breeding purposes at 240 days of age.

**Table 1: Diameter of the seminiferous tubules in relation to age, body weight and testicular weight in Deccani Rams**

Age (days)	Mean body weight (kg)	Testicular weight (gm)	External diameter of seminiferous tubule ( $\mu$ m)	Lumen diameter of seminiferous tubule ( $\mu$ m)
120 Days	12.50	6.95	114.84 $\pm$ 4.95	67.71 $\pm$ 3.94
150 Days	14.00	40.23	124.38 $\pm$ 3.72	59.40 $\pm$ 4.25
180 Days	15.75	42.02	147.21 $\pm$ 4.14	49.50 $\pm$ 5.74
210 Days	18.10	62.25	170.28 $\pm$ 3.97	55.44 $\pm$ 1.66
240 Days	20.25	110.35	180.18 $\pm$ 5.90	54.05 $\pm$ 3.76



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## Pathology Of Certain Uterine Abnormalities In Native Sheep

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The uterus is an important constituent of female reproductive system. It plays an important part not only in growth and development of normal lamb, it also becomes susceptible to various infections leading to pathological conditions at early post-partum stages.

McGreeken and Caldwell (1969) conducted post-mortem examination of a ewe that had not exhibited oestrus for 58 days and found that the left uterine horn was absent and that the same side was dominated by a large corpus luteum. Prabhakaran and Raja (1972) examined 1050 ewe (409 gravid) genitalia and reported macerated foeti (6), Pyometra (2), Mucometra (one), Perimetritis

(one), haematic mummification (3), and inter-cotyledonary haemorrhage (one case). O'Shed *et al* (1974) reported that normal oestrus cycles occurred in five ewes in which the left uterine horn was congenitally absent. Adams (1975) examined 485 genital tracts of ewes during March to May (when ewes not grazing oestrogenic pasture). Of these, 159 showed Macroscopic cysts in the uterus or cervix. 194 specimens were examined microscopically, of which 76 showed lesions of various organs including endometritis in 24%. The latter condition was associated with the presence of cysts in the uterus or cervix.

The objective of the present investigation was to examine the occurrence of

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pathological conditions of uterus encountered in slaughtered ewes.

### Materials and Methods

303 genitalia of nondescript sheep were procured from the local municipal small animal abattoir, Bareilly. Sheep were sacrificed during the early morning hours and the organs were removed and transferred to the laboratory immediately for the investigation in moist towels. Observations for ailments were well recorded. Small pieces of uterine tissue from 223 cases were fixed in 10% neutral formal saline for 24-72 hours.

### Results and Discussion

Of the 303 ewes, 42 (13.86%) were found to be suffering with gross pathological conditions of uterus, while others did not reveal any apparent abnormalities. Of the affected 42 animals, 59.52% showed the symptoms of endometritis. Blackish pigmentation of the mucosa was observed in one case (Fig. 1). The liquified foetal cases were the least. Metritis and anatomical anomalies were other conditions noted.

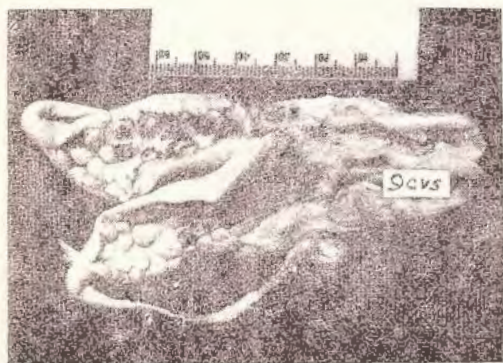


Fig. 1: Blackish pigmentation of the mucosa in uterus.

Of the three anatomical anomalies of uterus, one case was that of uterus unicornis (Fig. 2). The left uterine horn was congenitally absent, both the ovaries were present and blood supply was normal. The left oviduct

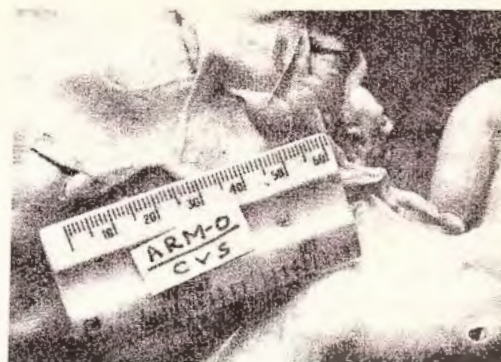


Fig. 2: A case of uterus unicornis. The oviduct is blind and stumpy.

was present in the proximal third only, forming a blind pouch towards the uterine end. Right horn had the foetus and appeared to be normal. On histo-pathological examination, the left ovary had a piece of bone. However, both ovaries looked normal macroscopically. The second case consisted of a fibrous membrane along the greater curvature of both the horns. The third case had thick fibrous inter-cornual bands (Table 1).

223 cases were studied for histo-pathological condition of uterus. 22 animals (9.8%) exhibited lesions (Table 2). Pigmentation due to melanin haemosiderine was found in 6.71% cases (Fig. 3). Of these,

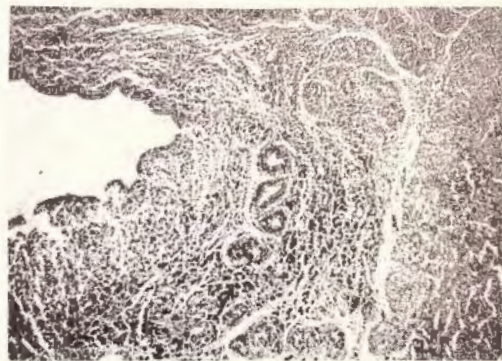


Fig. 3: Melanin pigmentation in uterine mucosa. H. & E. X 110.



3.13% revealed the pigment on one side and 3.58% in both uterine horns. Six cases revealed predominantly mononuclear cell infiltration with few neutrophils, if at all. Rarely, mild dilatation of uterine glands with

or without mild fibrosis was also observed in these cases. Such cases indicated the persistence of low grade infection. Adenomyosis (presence of uterine glands in the musculature) was observed in one case.

**Table 1: Percent incidence of various gross pathological conditions of uterus in Sheep (N=303)**

Sr. No.	Condition	No. of pathological cases.	% of pathological cases.
1.	Total cases	42	13.86
2.	Endometritis	25	8.25
3.	Metritis	8	2.64
4.	Anatomical anomalies	3	0.99
5.	Liquified foetus	1	0.33

N — No. of cases examined.

**Table 2: Percent occurrence of various histo-pathological conditions of uterus in Sheep (N=223)**

Sr. No.	Condition	Unilateral		Bilateral		Total	
		No.	%	No.	%	No.	%
1.	Pigmentation	7	3.13	8	3.58	15	6.71
2.	Subacute endometritis	1	0.44	5	2.22	6	2.66
3.	Adenomyosis	1	0.44	—	—	1	0.44
4.	Total	9	4.01	13	5.80	22	9.81

N — No. of cases examined.

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# Studies On Biochemical Composition Of Osmanabadi And Cross-Bred Buck Semen\*

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## ABSTRACT

Biochemical analysis of Osmanabadi buck semen revealed the initial fructose, total proteins and total lipid levels as  $486.511 \pm 32.34$  mg,  $4.69 \pm 0.20$  gm and  $341.225 \pm 13.38$  mg per 100 ml semen and  $404.70 \pm 23.55$  mg,  $3.54 \pm 0.25$  gm and  $275.54 \pm 14.28$  mg per 100 ml seminal plasma respectively. These constituents were also estimated in Osmanabadi crossbred bucks (50%). Highly significant variation was observed within individuals and also between pure bred Osmanabadi and their cross-breds for the levels of fructose in whole semen. Non-significant variation was observed within individuals and also between purebred Osmanabadi and their crosses for the levels of fructose in seminal plasma, proteins and lipid levels in semen and seminal plasma.

\* \* \*

On scanning the available literature, it is revealed that no information is available on biochemistry of cross bred buck semen. Osmanabadi is a dual purpose goat breed from Marathwada region. Studies on biochemical constituents of Osmanabadi and their cross-bred bucks may help to evolve appropriate dilutor for preservation of buck semen. If succeeded, it will help in undertaking AI programme in goat breeding on mass scale.

Fructose is claimed as chief nutrient for spermatozoa. Proteins are essential for buffer action, ionic equilibration and protective action. Lipids have important functions in cellular structure and in anaerobic metabolism of spermatozoa.

## Materials and Methods

Studies were undertaken at the 'Goat Unit' and the Department of Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, M.A.U. Parbhani. Only mature bucks with better body weight and libido were selected for study comprising of four Osmanabadi pure breed bucks, four Saanen  $\times$  Osmanabadi cross-bred (50%) bucks, two Alpine  $\times$  Osmanabadi cross-bred (50%) bucks. These bucks were trained for collection of semen by A.V. method.

Semen samples with optimum semen quality only were selected for study. Fructose determination was done as per 'Roe' (1934) with slight modification as cited by Mann (1964). Protein content was estimated by 'Biuret' method and total lipid content was estimated by 'Phosphovanilline' method. The data was subjected to 'analysis of variance' as per Snedecor and Cochran (1967).

## Results and Discussion

**Fructose:** Initial fructose level in Osmanabadi buck semen ranged from 255.907 to 736.980 mg per 100 ml with mean as  $486.511 \pm 32.34$  mg per 100 ml (Table I). The range of initial fructose is in approximation with the range 300 to 900 mg reported by Mann (1964) in buck semen.

Patil (1970) found mean initial fructose level of 611.94 mg per 100 ml semen in Malabari bucks. Barakat *et al* (1972) reported initial fructose level in bucks as  $820 \pm 38.69$  mg

\*A Part of the M.V.Sc. Thesis submitted to Marathwada Agricultural University, Parbhani — 431 402



Table 1: Details of estimated values of biochemical constituents of whole semen and Seminal Plasma of Osmanabadi pure bred and cross bred bucks.

CONSTITUENT & BREED	WHOLE SEMEN		SEMINAL PLASMA	
	Range	Mean	Range	Mean
<b>FRUCTOSE (mg/100 ml.)</b>				
Osmanabadi	255.907 to 736.98	486.511 $\pm$ 32.34	239.76 to 575.34	404.70 $\pm$ 23.55
Saanen X Osmanabadi	434.58 to 984.43	633.296 $\pm$ 34.02	302.27 to 714.69	404.708 $\pm$ 23.551
Beetal X Osmanabadi	423.05 to 805.76	602.89 $\pm$ 38.38	231.70 to 756.16	469.765 $\pm$ 46.775
Alpine X Osmanabadi	682.17 to 1205.76	795.30 $\pm$ 50.50	388.47 to 889.16	515.683 $\pm$ 46.66
<b>TOTAL PROTEIN (gm./100 ml.)</b>				
Osmanabadi	2.91 to 5.71	4.69 $\pm$ 0.20	2.50 to 5.98	3.54 $\pm$ 0.25
Saanen X Osmanabadi	3.23 to 7.10	5.27 $\pm$ 0.31	3.82 to 5.86	4.98 $\pm$ 0.16
Beetal X Osmanabadi	4.02 to 7.10	5.24 $\pm$ 0.31	2.98 to 5.26	4.04 $\pm$ 0.26
Alpine X Osmanabadi	3.69 to 5.85	5.03 $\pm$ 0.23	3.82 to 7.10	5.24 $\pm$ 0.34
<b>TOTAL LIPID (mg 100 ml.)</b>				
Osmanabadi	266.4 to 420.52	341.225 $\pm$ 13.38	162.67 to 357.83	275.54 $\pm$ 14.28
Saanen X Osmanabadi	297.61 to 425.52	352.93 $\pm$ 10.11	185.44 to 373.50	316.18 $\pm$ 12.39
Beetal X Osmanabadi	211.79 to 404.82	323.90 $\pm$ 21.07	211.97 to 352.46	291.29 $\pm$ 17.52
Alpine X Osmanabadi	282.08 to 404.85	343.10 $\pm$ 12.99	211.40 to 352.46	286.47 $\pm$ 16.35



per 100 ml of semen. Varshney *et al* (1977) observed initial fructose level of  $1294.50 \pm 15.10$  mg per 100 ml semen in Barbari bucks. Mittal and Ghosh (1980) estimated fructose level as  $795.92 \pm 10.72$  mg per cent in Parbatsar bucks. El-Sayed *et al* (1983) reported fructose level in whole semen of Baladi bucks as  $806.11 \pm 333.97$  mg per cent. Mean of initial fructose level in Osmanabadi buck semen is lower than the values reported by these workers in other breeds of bucks.

Initial fructose level in Osmanabadi buck seminal plasma was estimated as  $404.70 \pm 23.55$  mg per 100 ml. These values are lower than those of Igboeli (1979), who reported  $631.1 \pm 91.6$  and  $597.3 \pm 58.4$  mg per 100 ml seminal plasma in Zambian and Boer bucks respectively.

Highly significant statistical variation was observed in the levels of initial fructose in whole semen within individuals and also between pure bred Osmanabadi and crossbred bucks. However, variation of initial fructose values in seminal plasma was non-significant. Bishop *et al* (1954) observed non significant variation of concentration of initial fructose between the breeds but significant at 5% level between individual bulls.

**Total Proteins:** Total protein level in Osmanabadi buck semen estimated as

$4.69 \pm 0.20$  gm per 100 ml. (Table 1) is lower in Osmanabadi bucks as compared with 11.60 gm per 100 ml in Malabari bucks reported by Patil (1970).

Total protein level in the seminal plasma of Osmanabadi bucks was estimated as  $3.54 \pm 0.25$  gm per 100 ml which is in consonance with the values  $2.99 \pm 0.28$  gms reported by Dunder *et al* (1983) in Angora bucks. Variation of the values of protein in whole semen and seminal plasma was statistically non-significant within individuals and also between purebred Osmanabadi and cross bred bucks.

**Total Lipids:** Total lipid level in the Osmanabadi buck semen was observed as  $341.225 \pm 13.38$  mg per 100 ml and in seminal plasma it was  $275.54 \pm 14.28$  mg per 100 ml. Variation of the values of total lipids in whole semen and seminal plasma was statistically non-significant within individuals and also between pure bred Osmanabadi bucks and its crossbred bucks.

Total lipid values have not been reported so far by any of the research workers either in whole semen or seminal plasma of bucks.

### Acknowledgement

Authors are grateful to Dr. M.A. Ghafoor, Dean (Vet.) for providing necessary facilities and also to late Prof. Dr. M.N. Kulkarni for his valuable suggestions and guidance.

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## Changes In The Sperm Morphology During Their Passage Through Epididymis Of Goats

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As the spermatozoa emerge from the testes and begin to appear in the caput epididymis they exhibit no progressive movement and fertilizing ability. They acquire these properties gradually during the transit in the epididymis. The most striking morphological change associated with the passage is migration of proximal cytoplasmic droplet to the distal end of spermatozoa. This indicates the maturation process of spermatozoa which gives the ejaculated sperm their capacity for motility and fertility (Bedford, 1963).

### Materials and Methods

The epididymis along with testicles in pairs belonging to 6 local bucks were collected from slaughter house, Mhow, in a thermos flask containing ice. In the laboratory, the fluid was aspirated from ductuli efferentes, caput, corpus and cauda epididymis. For the study of middle piece and tail abnormalities of spermatozoa, the fluid was preserved with a drop of formal saline. The smears of the fluid obtained from different segments were prepared on clean micro slide.

The sperm head morphology was studied as per Williams (1920). The head

abnormalities were classified according to Lagerlof (1934). The presence of cytoplasmic droplets, acrosome defects, tailless heads, and abnormalities of middle piece and tail were studied under phase contrast microscope using wet preparations. The sperm abnormalities were classified according to Bane (1961) counting 200 sperms on each slide. Standard statistical method was applied for the analysis of the data (Snedecor and Cochran, 1967).

### Results and Discussion

The total number of abnormal sperm heads were maximum at the region of ductuli efferentes ( $20.97 \pm 3.28$ ) followed by a significant fall ( $P < 0.01$ ) at the region of caput ( $15.64 \pm 2.25$ ), corpus ( $10.98 \pm 2.06$ ) and cauda ( $11.31 \pm 1.78$ ) epididymis. The number of tailless heads, middle piece and acrosome defects did not vary significantly in the segments of the reproductive tract.

The sperm tail defects were minimum at ductuli efferentes ( $2.32 \pm 0.73$ ) followed by significant increase ( $P < 0.01$ ) at caput ( $4.32 \pm 1.13$ ), corpus ( $9.65 \pm 1.54$ ) and cauda ( $15.99 \pm 3.08$ ) epididymis.



The number of proximal cytoplasmic droplets decreased significantly ( $P<0.01$ ) from ductuli efferentes/caput epididymis ( $189.00\pm1.12/180.33\pm3.48$ ) to corpus/cauda epididymis ( $26.66\pm1.11/8.33\pm0.95$ ). The trend was reverse in the occurrence of distal cytoplasmic droplets, as their number increased significantly ( $P<0.01$ ) from ductuli efferentes/caput epididymis ( $3.00\pm0.65/$

$6.00\pm0.73$ ) to corpus/cauda epididymis ( $156.33\pm5.20/184.66\pm4.92$ ).

The findings in the present study are in agreement with the work of other investigators in the goat (Curry and Vinha, 1982) and Rao, (1971) and Rao *et al* (1980) in cattle, who attributed reduction in abnormal heads at caput epididymis due to its great resorptive power.

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## Plasma Progesterone Concentration During Oestrous Cycle In Goat

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### ABSTRACT

Plasma progesterone concentrations in German dwarf goats of mixed inheritance were analysed by radio-immunoassays (RIA). The sensitivity of the assay was 0.15 ng/ml. Blood samples were collected at 3 days intervals from onset of oestrous till next oestrous in cyclic goats. The progesterone

values in plasma were under 1 ng/ml immediately before the sexual season (in August) and during oestrous. The values increased from 0.25 ng/ml on day 0 to 10.3 ng/ml on day 12th. Thereafter, the values declined and were below 1 ng/ml on day 21 of the cycle.

\* \* \*

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The superovulatory response after administration of gonadotrophins during early (Wani *et al.*, 1989) and late (Moore and Eppleston, 1979) Luteal phase were better than their administration during mid Luteal phase (Ott *et al.*, 1979). The knowledge of progesterone concentrations during different phases of oestrous cycle was thus thought to be helpful in selection of potential donors. Therefore, the present study was undertaken, to analyse progesterone concentrations in blood plasma of cyclic goats.

### Materials and Methods

14 German dwarf goats of mixed inheritance were used in this experiment. The animals were maintained under identical conditions and kept as single or in small groups of 2-3 animals per box. Heat detection was undertaken by parading an aproned buck in each box twice daily. The blood samples were collected from jugular vein of cyclic goats on 0, 3, 6, 9, 12, 15, 18, 21 days of estrous in heparinized (1 mg Vetren<sup>®</sup>/ml blood Promonta, Hamburg) plastic tubes and centrifuged at 4°C by 5000 g for 20 min. The separated plasma was stored in 1 ml plastic tubes at -20°C. Progesterone concentrations were measured by RIA at Endocrinology and Laboratory, Clinic for Bovine Gynaecology, Veterinary School, Hannover. The sensitivity of the assay was 0.15 ng/ml.

### Results and Discussion

Plasma progesterone concentration values immediately before the onset of breeding season (August) and during oestrous were low in all the goats. These increased gradually to peak value (10.3 ng/ml) around day 12th of oestrous cycle. Thereafter, they declined and reached basal low values around day 21 in cyclic goats (Table 1). These observations are comparable to those reported by Chemineau *et al.* (1982) and Lucaroni *et al.* (1980).

The plasma progesterone values ranged between 1.6-6.5 ng/ml during early and late phases of oestrous cycle (3-6 day or 15-21 day of the cycle, Day of oestrous = DO), but were much higher (8.8-13.3 ng/ml) during mid cycle (Day 9th-12th). Better ovulation rates were observed in goats when superovulations were attempted during early and late phase of oestrous cycle (Wani *et al.*, 1984, 1989; Moor *et al.*, 1980), than during mid cycle (Ott *et al.*, 1979). The high progesterone profile during this phase as observed in this study may perhaps explain the low and varied superovulatory response during midcycle stimulations in these goats as reported (Wani *et al.*, 1980).

The progesterone assay may thus be useful in selection of potential donors to avoid varied superovulatory response, which is one of the strongest limitation of embryo transfer programme (Armstrong *et al.*, 1983).

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**Table 1: Plasma progesterone concentrations during oestrous cycle.**

Item	Time of blood collection								
	Before onset of breeding season (August)	During oestrous (Day 0) = DO	Oestrous cycle days						
			D3	D6	D9	D12	D15	D18	D21
No. of animals	14	10	8	6	3	3	3	3	3
Plasma progesterone concentration (ng/ml)									
X	0.22	0.25	1.64	6.2	8.8	10.3	6.5	3.3	0.19
S	0.05	0.09	0.93	1.6	2.7	3.8	3.1	1.5	0.04
Range	0.15-0.31	0.15-0.38	0.80-27	3.7-7.9	6.7-11.9	6.6-14.3	4.6-10.1	2.6-5.6	0.15-0.21

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## Superovulation And Synchronization Of Estrus In Goats

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### ABSTRACT

Oestrus in 16 goats was synchronised with daily I.M. injections of 12 mg progesterone for seven days. Superovulation in 8 goats was carried out with injections of 2 doses of PMS

gonadotrophin 1000 IU, 24 hours after withdrawal of progesterone therapy and 400 IU on the following day and chorionic gonadotrophin 1000 IU given intravenously 12 hours after the onset of oestrus.

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All the goats showed estrus 1 to 2 days of the final PMSG injection. An average of 13.87 follicles developed in the ovaries with an average of 10.62 ovulation points. The ovulation percentage was 76.58% with 3.25 follicles found unruptured.

\* \* \*

Synchronization of oestrus is carried out to bring the animals in oestrus within a short period of time of 1-2 days, so that they could be mated together for planning the parturitions. It is also necessary for transfer of embryos to have the donor and recipients at the same stage of estrus. By superovulation, we can obtain large number of ova from a single donor for transfer in a large number of recipients.

PMSG was first used for multiple pregnancies in sheep by Loginova and Lopyrin (1938). Superovulation was induced in goats by Van Rensburg (1964), Suh *et al* (1975), Ahmed and Maurya (1981), Agrawal *et al* (1982), Armstrong *et al* (1982) and Pandiya and Rathor (1986).

Suh *et al* (1975) induced superovulation in goats by progesterone treatment followed by 1000 IU PMS and 100 IU HCG. Armstrong *et al* (1982) induced ovulation in goats before onset of breeding season by PMS and synthetic progestogen.

#### Materials and Methods

16 non-descript goats of 2-3 years of age were estrus synchronised with daily intramuscular injections of 12 mg progesterone (Progesterone injection, Lyophilic Labs., Bombay) for seven days, out of which superovulation was induced in 8 goats by I.M. injection of 1000 IU of PMSG (Folligon, Intervet International, Holland) twentyfour hours after withdrawal of the progesterone therapy, followed by a second dose of 400 IU of PMSG on the following day.

The goats were detected in estrus with the help of an approned buck of good libido.

Chorionic gonadotropin (Chorulon, Intervet International, Holland) 1000 IU was injected intravenously twelve hours after the onset of estrus. The goats were mated two times with bucks of proven fertility, 4-6 hours after the onset of oestrus. They were laparotomised after 72, 96 and 120 hours after onset of oestrus for collection of embryos in another series of experiments and the ovulation points and matured graafian follicles were counted on both the ovaries.

#### Results and Discussion

An average of 13.87 (range 9-20) follicles developed on both ovaries of the goats and an average of 10.62 (range 3-18) ovulation points were observed. Matured follicles indicated 76.58 percent ovulations (Table 1).

The synchronization of estrus in goats was 100% which is comparable with the results of Van Rensburg (1964) using 20 mg progesterone for five days, Marincowitz (1967) with 25 mg for 3 days and Ahmed and Maurya (1981) who used hydroxyprogesterone caproate 10 mg for 6-14 days. Suh *et al* (1975) had used only one dose of PMSG while two doses of PMSG were used in the present investigation.

The ovulation rate of 10.62 in the present investigation is quite comparable with that obtained by Armstrong *et al* (1982) and Ahmed and Maurya (1981).

The ovulation percentage of 76.58% in the present investigation is higher than that of 73.4% reported by Nishikawa *et al* (1963) and 71.14% reported by Pandiya and Rathore (1986), respectively.

The average number of unruptured follicles was 3.25 which is lower than the average of 4.55 follicles observed by Pandiya and Rathore (1986). A higher dose of LH might be necessary for rupturing all the matured follicles.



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**Table 1: Superovulatory response of PMSG and HCG regimen on goat ovaries.**

Sl. No.	Animal No.	Total mature follicles developed		Total	Total ovulation points observed		Total	Overall p.c. of ovulations
		Lt. ovary	Rt. ovary		Lt. ovary	Rt. ovary		
1.	21	4.0	5.0	9.0	1.0	2.0	3.0	33.33%
2.	28	8.0	12.0	20.0	6.0	10.0	16.0	80.00%
3.	38	10.0	10.0	20.0	10.0	8.0	18.0	90.0%
4.	22	9.0	7.0	16.0	8.0	6.0	14.0	87.5%
5.	35	6.0	5.0	11.0	4.0	4.0	8.0	72.72%
6.	32	4.0	7.0	11.0	3.0	4.0	7.0	63.63%
7.	29	6.0	9.0	15.0	4.0	6.0	10.0	66.66%
8.	34	4.0	5.0	9.0	4.0	5.0	9.0	100%
Total	8	51.0	60.0	111.0	40.0	45.0	85.0	76.58%
		Mean		13.87			10.62	

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## Hormonal Induction Of Lactation And Histomorphology Of Mammary Glands In Prepubertal Goats

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The work on induction of lactation in adult goats has been reported by some workers after administration of different proportions of estrogen and progesterone for varied lengths of time (Cowie *et al*, 1965 a,b; Montigny *et al*, 1981 and Almlid, 1983), but apparent lack of information in this regard in prepubertal goats prompted this investigation.

15 prepubertal goats of 2 1/2 to 5 months of age and 4 to 10 kg in weight were included in this study, 5 of which were ovariectomized one month before the experiment. The five ovariectomized and five intact goats comprised the experimental group and five intact goats served as control. The experimental goats were given seven injections of stilbesterol dipropionate (Vetestrol, May and Baker) and Progesterone (Lyophilic Lab., Bombay) @ 0.25 mg and 0.75 mg/kg body weight respectively, subcutaneously, on alternate days. Prednisolone (Hostacortin 'H', Hoechst) 0.4 mg/kg body weight was injected intramuscularly daily for 3 days, 24 hours after the last hormonal injection. Massage of the mammary glands was started after the third dose of the hormones to stimulate lactogenesis. One month after the treatment, biopsy samples from the mid region of the mammary glands were collected surgically from all the goats. The tissues were fixed in 10% buffered formalin and processed by routine paraffin tissue technique. Sections of

5  $\mu$ m thickness were cut and stained with haematoxylin and eosin (Lillie and Fullmen, 1976). The milk yield was recorded weekly for a period of 13 weeks.

### Results and Discussion

The experimental goats responded favourably to the treatment, resulting in an increase in the size of the mammary glands (Fig. 1-3). Lactation started from 13th to 15th day from the start of the treatment. The peak average weekly milk production (495.2 ml) was attained earlier (in 5th week) by the intact animals as compared to the peak yield (427.0 ml) attained (in 7th week) by the ovariectomized goats. The lower quantity of milk produced by the treated goats in this experiment could be attributed to the lower ages and body weights of the animals. The



Fig. 1: Rudimentary mammary glands (arrows) in prepubertal control goat.

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Fig. 2: Well developed mammary glands in normal treated prepubertal goat.



Fig. 3: Well developed mammary glands in ovariectomized prepubertal goat.

data indicate that the yield was relatively higher throughout the observation period in intact treated goats than in ovariectomized ones. However, the difference was statistically nonsignificant. This is in agreement with the findings of Sud (1972) in heifers. All the treated animals showed behavioural signs of oestrus within 2-3 days of the first stilbestrol and progesterone injection which persisted for about 3-4 days.

The histological examination of mammary glands of the control goats revealed an abundance of connective tissue. Various sized accumulations of adipose cells were likewise present. Few ill-developed alveoli and ducts were discernible within the lobule. Under the influence of hormonal treatment,

there was a decrease in the connective tissue which was replaced by glandular tissue. The alveoli became distended and lined with single or two layered cuboidal to columnar epithelial cells resting on a distinct basement membrane. The alveoli were round, oval and irregular in shape. The connective tissue between the alveoli was loose areolar. The lobular ducts were lined with simple cuboidal or low columnar epithelial cells. This was in conformity with the findings of Parmer *et al* (1987) in lactating adult goats. Thus, it is concluded that the prepubertal goats and even Kids of 2 1/2 months of age could be successfully induced to lactation by the hormonal treatment. The presence of ovaries is not indispensable for such induction and maintenance of lactation in this species.



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## Ovarian Response After Repeated Superovulation In Cross-bred Cows\*

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### ABSTRACT

Six cross-bred repeat breeder cows were superovulated by administering a combination of PGF<sub>2</sub> alpha and PMSG. Considerable variation in superovulatory response was observed after first superovulation treatment. Repeat superovulation at an interval of eight to ten weeks was performed in all the cows using the same treatment regimen on six successive occasions. The ovarian response was found to decrease significantly ( $P < 0.01$ ) after first two superovulatory treatments in all the cows. All cows except one developed varying numbers of anovulatory follicles. In one cow where the

ovarian response was judged to be very poor, these follicles were palpable following all the six superovulatory responses.

\* \* \*

Superovulation is a procedure by which numerous oocytes can be salvaged and made to ovulate. The success of egg transfer technique depends largely on reliable methods for the induction of superovulation in order to harvest large number of normal fertile eggs or embryos. The most common method is the stimulation or follicular growth and ovulation with the help of PMSG hormone. Prostaglandin F<sub>2</sub> alpha has been extensively used in cattle for the control and

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synchronisation of estrous cycle because of its luteolytic properties. To induce superovulation, prostaglandin F<sub>2</sub> alpha in combination with serum ganadotrophin has been preferred over progestagens due to its ease of administration and for eliciting better ovarian response. However there are limitations on the number of eggs which may be produced during one superovulatory treatment and the procedure needs to be repeated to recover maximum number of eggs from individual cows. Ovarian response after repeated superovulation has not been extensively studied and the results obtained so far are inconclusive. Therefore, present investigation was carried out with the two-fold objectives: (1) To study the superovulatory response in cross bred cow following administration of PMSG and PGF<sub>2</sub> and (2) To monitor the ovarian response after repeated superovulation with PMSG and PGF<sub>2</sub> alpha.

### Materials and Methods

The present investigation was carried out on six cross bred (Holstein-Friesian × local) repeat breeder cows maintained at Ranchi Veterinary College, Birsa Agricultural University, Ranchi. Based on gynaeco-clinical examination and A.I. records, these animals were diagnosed to be repeat breeders with regular estrous cycle for the last six months or more after calving. The cows were healthy with apparently normal genital tract and ovaries. Their average age was between 4-8 years and had calved 2-3 times previously. They were maintained in a separate barn under iso management conditions. Feeding was carried out as per the N.R.C. standard and they were allowed grazing for 5-6 hours during the day.

The superovulation regimen consisted of two intramuscular injections of 25 mg PGF<sub>2</sub> alpha (Lutalyse, UpJohn) at 10 days interval.

Forty eight hours prior to second injection of Lutalyse, 1500 I.U. PMSG (Antex, Leo) was administered I.M. The cows were checked for symptoms of estrus and were bred by A.I. with chilled semen from bulls of documented fertility, twice at 48th and 72nd hours after second injection of PGF<sub>2</sub> alpha. Ovarian response was monitored by rectal palpation between day 6 and 8 (estrus, day 0). The response was graded on the basis of number of palpable corpora lutea on both the ovaries. The response was rated as good (more than 12 corpora lutea) moderate (6 to 12 corpora lutea) and poor (1 to 5 corpora lutea) as per Donaldson (1985). The ovaries were considered as having refractory stimulation when there were no palpable corpora lutea. Repeat superovulation was performed at 8 to 10 weeks interval for six successive occasions with identical drug and dose regimen.

### Results and Discussions

Out of six cows, two cows (no. 5 and 6) showed good response (>12CL) whereas, cow nos. 1, 2 and 3 showed moderate response (6-12 CL) and in cow no. 4 the response was nil, following first superovulatory treatment (Tables 1, 2). These are in accordance with Gupta *et al* (1983) who found that three out of eleven cows had shown good response. The ovarian response was maximum (18 CL) in cow no. 6 following first superovulatory treatment. These results suggest that the ovarian response was quite variable between the cows. Several workers have reported variation in response after superovulatory treatment with PMSG (Becze *et al*, 1978; Haupt, 1979). Various factors including individual variation (Betteridge, 1977; Maria *et al*, 1977; Kosugiyama *et al*, 1979) batch of PMSG used (Betteridge, 1977 and Vlachov *et al*, 1983), season of the year and age of the animal (Haupt, 1979) and endogenous hormonal milieu (Haupt, 1979 and Sreenan *et al*, 1980) have been suggested as probable reasons for the variation in response.



Saumande *et al* (1984) opined that variability of response was a consequence of the difference in the sensitivity and follicular growth among animals. Variation in the response to the treatment was noted depending on the age and body weight of the animal by Hauptat (1979). He further observed that the level of progesterone at the time of PMSG administration accounted for 16% of the variation in ovulation rate. Cows with higher milk progesterone levels reacted significantly better than those with lower level. Numerically cow no. 5 had the highest number of corpora lutea (52/227) and was considered to have shown the best response. In addition, it had not developed a single anovulatory follicle (Table 1). The response was poorest in cow no. 4, wherein only 13 corpora lutea could be palpated after six treatments with a high percentage (19.63%) of anovulatory follicles. A total of 227 corpora lutea could be palpated after six repeated superovulation in six cows (Table 2). Ovarian response after six repeated superovulatory treatments was significantly ( $P<0.05$ ) higher in cow nos. 5 and 6. The overall response was moderate following 1st and 3rd superovulatory response and poor after 4th to 6th treatments (Table 2). A consistent decrease in response was marked in all the cows after 2nd or 3rd superovulatory treatments except in cow no. 4, where the initial response was nil, improved slightly after 2nd to 5th superovulatory treatments and again declined after 6th treatment. As compared to the first two treatments the ovarian response decreased significantly ( $P<0.01$ ) upto the 6th treatment (Table 3). The ovarian response was observed to decrease after repeated superovulatory treatment in earlier studies as well (Saumande and Chupin, 1977; Donaldson and Perry, 1983 and Schilling *et al*, 1984) and the reason for decrease in response has been ascribed to

the development of refractoriness (Jainudeen *et al*, 1966). Willet *et al* (1953) suggested that refractoriness developed due to exhaustion of primary follicles or varying degrees of damage to the ovaries due to treatment or surgery. Alkamali *et al* (1984) observed the same decreasing trend in ewes after repeated superovulation. Labbadeh *et al* (1980) reported that refractoriness to repeated gonadotrophin injections did not develop within 3-4 months period required for the four superovulatory treatments in their experiment and the decreased response of the cows in the 3rd and 4th superovulation period was attributed to a short recovery time from the previous superovulation. Schilling *et al* (1984) found decrease in response after the fourth treatment.

Almost all the cows under the present experiment developed certain numbers of anovulatory follicles following all the six treatments except cow no. 5 (Table 1). A total 107 such follicles were palpated between day 6 to 8. The percentage of such follicles was high after 1st and 2nd superovulatory treatments as compared to subsequent four treatments (Table 2). Cow no. 2 developed the highest percentage of such follicles followed by cow nos. 1, 6, 4 and 3, whereas cow no. 5 did not have a single anovulatory follicle (Table 1). These follicles disappeared spontaneously and were not palpable on rectal examination after a week's time. Anovulatory follicles have also been reported by Nikoyan (1982), Silvas *et al* (1981) and Kuttner (1978) after PMSG treatment for superovulation. It was suggested by Sreenan (1978) that these follicles persisted due to their failure to bind sufficient quantity of LH during preovulatory surge. Moyaert *et al* (1985) concluded that the number of unruptured follicles could be reduced significantly by the use of anti-PMSG serum or monoclonal PMSG antibody injected at the time of estrus.



**Table 1: Ovarian response in individual cows after six repeated PMSG treatments**

Cow No.	Ovarian response				Total		Percent	
	LO		RO					
	CL	AF	CL	AF	CL	AF	CL	AF
1	16	12	24	10	40 (6.7)	22 (3.7)	17.62	22.56
2	15	15	16	14	31 (5.1)	29 (4.8)	13.66	27.10
3	21	5	20	6	41 (6.8)	11 (1.8)	18.06	10.28
4	7	11	6	10	13 (2.1)	21 (3.5)	5.73	19.63
5	17	—	35	—	52 (8.7)	—	22.91	—
6	24	12	26	12	50 (8.3)	24 (4.0)	22.03	22.43

CL = Corpora Lutea; AF = Anovulatory follicle; LO = Left ovary; RO = Right ovary.  
Values in parentheses indicate average number of corpora lutea/anovulatory follicles in each cow after one superovulatory treatment.

**Table 2: Ovarian response following repeated PMSG treatment.**

Treatment No.	Ovarian response				Total		Percent	
	Left ovary		Right ovary					
	CL	AF	CL	AF	CL	AF	CL	AF
I	31	16	34	16	65 (10.8)	32 (5.3)	28.63	29.91
II	30	8	31	12	61 (10.2)	20 (3.3)	26.87	18.70
III	14	8	22	4	36 (6.0)	12 (2.0)	15.86	11.21
IV	12	6	16	7	28 (4.6)	13 (2.1)	12.33	12.15
V	9	11	14	6	23 (3.8)	17 (2.8)	10.13	15.89
VI	4	6	10	7	14 (2.3)	13 (2.1)	6.17	12.15

CL = Corpora lutea; AF = Anovulatory follicle

Number of parentheses indicate average number of corpora lutea/anovulatory follicle after superovulatory treatment.



**Table 3: Analysis of variance for ovarian response**

Source of variation	d.f.	mss	F
Between cows	5	34.23	5.86**
Between treatments	5	72.09	12.34**
Error	25	5.84	

\*\*P<0.01

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## Standardisation Of Semen Filtration Technique Through Different Media To Improve Its Quality

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### ABSTRACT

Efficiency of Glasswool and Sephadex gel to filter semen for improving the quality was studied. When samples were filtered through glasswool in Pasteur Pipette columns of 1 and 2 cms. length, there was significant improvement in sperm motility from 61 to 82% and live sperms from 70 to 88%. There was a decrease in the abnormal sperm count by 57 to 63% and sperm concentration by 23 to 39% in 1 cm. and 2 cms. columns respectively. The filtered semen samples on dilution and storage in the refrigerator were able to sustain reasonable viability up to eight days. 1 cm. glasswool column was found to be better than 2 cms. column by virtue of short duration of filtration period- 5 min vs 13 min and higher recovery of the volume 85% vs 45% and maintaining higher motility during storage.

Three varieties of columns with Sephadex G-200 were tried- (i) 500 mg in 22 mm column of gel bed length 5.5 cms (ii) 250 mg in 11 mm column of bed length 5 cms (iii) 250 mg in 22 mm column of bed length 3 cms. There was improvement in sperm motility and live

sperm count, with filtered semen. Column (iii) had higher motility i.e., 34 vs 32 and 22% and live sperm 23 vs 16 and 14% respectively. The time taken for filtration was short i.e., 5 min vs 13 and 14 min. In addition, the elute volume was 25% more when compared to the other two varieties. The findings suggest that by filtering semen in 22 mm column containing 250 mg of Sephadex G-200, the quality of semen can be improved.

It is a common practice that in most of the semen processing laboratories the entire ejaculate is taken and diluted after preliminary evaluation for volume, consistancy and motility. These ejaculates comprise of heterogenous collection of cells varying in morphology, live and dead percentage, degree of motility and maturation. In the process of collection, dilution and preservation, the number of dead sperms increase resulting in decrease of fertilizing capacity. It is necessary to consider whether any form of sperm selecting method is required so that more vigorous spermatozoa have greater opportunity of reaching the site of fertilization. Studies undertaken by Baker



and Degen (1972) and Mortimer (1977) strongly suggest that less viable spermatozoa fail to reach the site of fertilization. Graham (1976) and Fernandez (1986) attempted to filter the spermatozoa and observed that there was significant decrease in the number of dead spermatozoa. The present investigation is aimed to evolve a method to separate viable sperm from non-viable one in the initial stage, process them and study the keeping quality.

### Materials and Methods

Holstein Friesian Bulls maintained at Southern Regional Station of National Dairy Research Institute, Bangalore, were used for the study. The bulls were in the age group of three to five years and were maintained under normal managerial conditions. One collection a week with consecutive ejaculates were taken from each bull using A.V. technique. Of the two ejaculates, the one with lower motility was taken for the experiment.

**Glasswool column:** Sterilised Borosilicate glasswool was filled in Pasteur Pipette. The quantity of glasswool taken was 100 mg and 50 mg and length of the column was two cms and one cm respectively. Immediately after collection of the semen, a known quantity was passed through the column and the elute volume and time taken for filtration was noted.

**Sephadex Gel column:** After preliminary trials with Sephadex gel of different grades, Sephadex G-200 was found to be suitable. Since neat semen could not pass through the gel, the problem was overcome by diluting with equal quantity of Tris buffer and with this the filtration was rapid.

Three varieties of columns with sephadex G-200 were tried: (i) 500 mg in 22 mm column of bed length 5.5 cms (ii) 250 mg in 11 mm column of bed length 5 cms (iii) 250 mg in 22 mm column of gel bed length 3 cms. Observations were made for the difference in

the volume, sperm concentration, percentage of abnormal sperms and the time taken for the filtration. The filtered sample was extended in Tris buffer containing egg yolk and kept stored in the refrigerator. The motility and percentage of live sperms were recorded daily up to a period of eight days. The control samples were not filtered but they were diluted in the same ratio and similar observations were made.

### Results and Discussions

**Glasswool as filter media:** When the semen samples were filtered through glass wool column of 1 and 2 cms length, the 1 cm column was found superior to 2 cms column with respect to the volume of semen filtered i.e., 3.42 ml vs 1.8 ml and time taken for filtration, 5.17 min vs 13 min (Table 1). There was a decrease in sperm concentration by 23 to 39% and abnormal sperm count by 57 to 63%. When filtered semen sample was diluted and examined for motility and live sperm percentage, it was observed that there was significant improvement in the initial sperm motility from 61 to 80% and live sperms from 70 to 87% (Fig. 1). Further it was observed that on storage in the refrigerator, the samples were able to sustain reasonable viability up to a period of eight days. Brown and Johnson (1958) and Krishnamurthy *et al* (1953) suggested the use of glasswool for filtering semen and the present findings are in agreement.

**Sephadex as filter media:** When Sephadex G-200 columns of (i) 22 mm diameter with 500 mg gel bed length of 5.5 cms (ii) 11 mm diameter with 250 mg gel bed length of 5 cms (iii) 22 mm diameter with 250 mg gel bed length of 3 cms were used, the column type (iii) with 22 mm diameter having 250 mg gel bed of 3 cms was found more efficient when compared to the other two columns i.e., the volume recovered was 70% against 44 and 41% and time taken for filtration was 5.65 min vs 13.6 and 12.8 min (Table 2).



FIG. 1: Motility and live sperm percentage following filtration in Geeswood

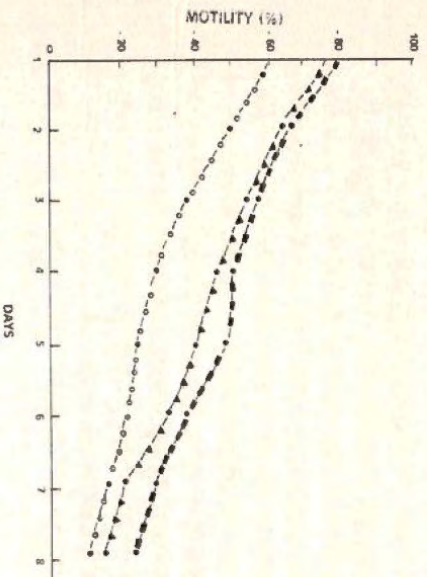


FIG. 2: Motility and live sperm percentage following filtration in Sephadex

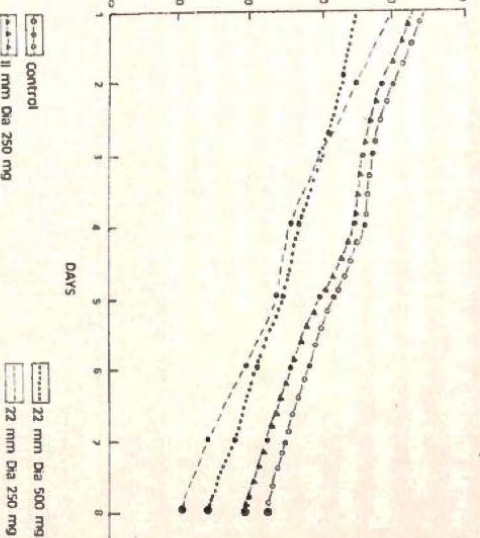
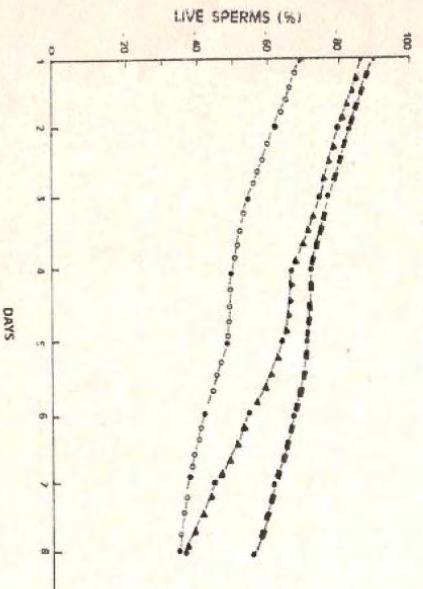
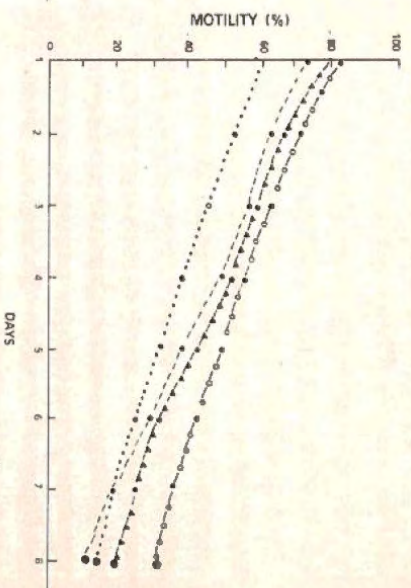




Table 1: Results of filtration of semen through glasswool columns in pasteur pipette

Particulars of Column	Volume in ML		Time taken (In minutes)	Sperm concentration (In millions/ml)		Percentage of Abnormality	
	Pre Filtration	Post Filtration		Pre Filtration	Post Filtration	Pre Filtration	Post Filtration
I Glasswool 100 mg Length = 2 cms N = 6	4.0	1.8 ±0.09	13.00 ±0.81	1583.3 ±165.5	966 ±65.5	13.16 ±0.79	4.83 ±0.04
II Glasswool 50 mg Length = 1 cm N = 20	4.0	3.42 ±0.13	5.17 ±0.28	1426 ±93.28	1093 ±82.90	11.2 ±0.86	4.85 ±0.33
Control N = 26	1.0	—	—	1426	—	11.2 ±0.86	—

Table 2: Results of filtration of semen through sephadex gel columns.

Particulars of Column	Volume in ML		Time taken (In minutes)	Sperm concentration (In millions/ml)		Percentage Abnormality	
	Pre	Post		Pre	Post	Pre	Post
Column (i) GEL = 250 mg Dia = 11 mm length = 5.0 cms N = 5	8.0	3.5 ±0.52	13.66 ±0.67	1260 ±150.30	530 ±75.16	12.86 ±1.02	7.54 ±0.55
Column (ii) GEL - 500 mg Dia = 22 mm length = 5.5 cms N = 5	8.0	3.3 ±0.2	12.8 ±0.86	1366 ±57.69	680 ±51.20	14.05 ±0.94	8.65 ±0.45
Column (iii) GEL = 250 mg Dia = 22 mm length 3 cms N = 20	8.0	5.5 ±0.6	5.65 ±0.72	1366 ±57.59	1041 ±45.61	13.87 ±0.76	6.07 ±0.15
Control N = 30	2.0	—	—	1366	—	13.87	—



It was observed that there was an increase in the initial motility from 61 to 75, 81 and 82% for the three types of columns used (Fig. 2). Similarly the increase in live sperms count was 70 to 80, 82 and 87% in the three columns tried. In addition it was observed that, when the semen was filtered through 22 mm column containing 250 mg gel, it retained its higher motility, live sperm and viability up to eight days when compared to column (i) and (ii). Heuer (1982) has suggested the use of

Sephadex as a filter media to improve the quality of semen and the present findings are in agreement with his results.

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## Spermogram And Cytomorphology Of Spermatozoa In Relation To Freezability Of Holstein-Friesian Semen

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#### ABSTRACT

Physical characteristics of semen and cytomorphology of spermatozoa of 20 Holstein-Friesian bulls (HF) in relation to their freezability was studied. The ejaculate volume, initial motility, sperm concentration per ejaculate and live sperm count did not change significantly in Group II (medium freezable) and III (poor freezable) ejaculates

when compared with Group I (good freezable) bulls. The cytomorphology of spermatozoa revealed non-significant increase in sperm head abnormalities, acrosomal abnormalities, mid-piece defects and proximal cytoplasmic droplets in Group II and III ejaculates of HF bulls.

\* \* \*



Electron microscopic picture of the bovine spermatozoa pre and post freezing in liquid nitrogen was studied by Healey (1969). The poor freezable ejaculates, unsuitable for freezing, exhibiting acrosomal damage are reported by Veres *et al* (1972 a,b). Mohanty and Dugwekar (1981) found non-significant difference between the bull producing semen of normal and poor freezability in sperm resistance to cold shock. The present communication deals with the spermiogram and cytomorphological characters of Holstein-Friesian (HF) spermatozoa in relation to freezability of ejaculates.

#### Materials and Methods

Semen was collected from 20 HF bulls, twice a week by using artificial vagina at Semen Bank, Patiala. 10 to 12 ejaculates from each bull were collected to study the physical characteristics of semen i.e. volume, mass activity, initial motility, sperm concentration and live sperm count as per standard techniques. The sperm morphology was studied by using Giemsa stain and under phase contrast microscope. The ejaculates of bulls were separated into three groups according to the freezability percentage (Pangawkar *et al*, 1988). The bulls exhibiting >85% ejaculates freezable were comprised under Group I (good freezable), between 65 to 85% in Group II (medium freezable) and <65% ejaculates freezable in Group III (poor freezable). The results were analysed statistically as per Snedecor and Cochran (1967).

#### Results and Discussions

The mean volume of semen of HF bulls (Group I) was recorded to be  $4.78 \pm 0.14$  ml (Table 1), which was supported by Kumar (1975) and Mohanty and Dugwekar (1981). The non-significant increase of ejaculate volume in Group II and III bulls might be due to individual variations. The mass activity of Group I was similar to those reported by Kupferschmied (1975) and Mohanty and

Dugwekar (1981). The significant lower mass activity in Group II and III bulls might be ascribed to lower sperm concentration. The observed mean initial motility (Group I) corresponded to the values reported by Taha (1974). The initial motility was almost unchanged in Group II and III ejaculates. The mean sperm concentration in Group I bulls was enumerated to be  $1221.22 \pm 65.31$  millions per ml which was close to the figures reported by Holy (1971) and Roy and Ansari (1973). The sperm concentration was lower in Group II and III though the differences between the groups were statistically non-significant. The total sperm per ejaculate was found to be lower in Group II and higher in Group III as compared to Group I ejaculates. The mean live sperm count was in conformity with the results obtained by Foote *et al* (1977) and Satyanarayana Raju *et al* (1982) in HF bulls. The live sperm percentage was comparable in Group II and III ejaculates.

The cytomorphology of spermatozoa in the ejaculates of Group I, II and III revealed that the freezability of ejaculates decreased with increase in the incidence of head and acrosomal abnormalities, though the differences in mean values of the abnormalities between the three groups were found to be non-significant. The incidence of free heads, head defects, acrosomal abnormalities, mid-piece, tail defects and proximal cytoplasmic droplets in HF bulls appeared to be quite low (Table 2). Findings of Saacke (1970), Rajkonwar (1979) and Mohanty & Dugwekar (1981) corroborate the present observations. Higher percentage of acrosomal abnormalities in fresh ejaculates of medium and poor freezable groups might suggest that incidence of acrosomal deformity could be indicative of lower freezability of semen samples, since Veres *et al* (1972 a,b) recorded alterations in the acrosomes of spermatozoa in the ejaculates which did not freeze satisfactorily.



**Table 1: Semen characteristics in different freezability groups of Holstein-Friesian bulls**  
(Mean  $\pm$  S.E.)

Freezability group	Number of Bulls	Volume (ml.)	Mass activity (0-3)	Initial motility (%)	Sperm conc. (millions/ml.)	Sperm conc (millions/ejaculate)	Live sperm count (%)
I	9	4.78 $\pm$ 0.14	2.61 $\pm$ 0.05	73.22 $\pm$ 0.77	1221.22 $\pm$ 65.31	5892.96 $\pm$ 296.78	86.20 $\pm$ 1.51
II	4	-5.30 $\pm$ 0.30	2.30 $\pm$ 0.07	71.87 $\pm$ 1.47	956.00 $\pm$ 87.10	5372.75 $\pm$ 705.29	87.48 $\pm$ 1.64
III	7	-5.11 $\pm$ 0.39	2.60 $\pm$ 0.07	72.57 $\pm$ 0.86	1194.57 $\pm$ 112.45	6260.50 $\pm$ 1009.73	85.35 $\pm$ 1.07

**Table 2: Spermatozoan morphology in different freezability groups of Holstein-Friesian bulls**  
(Mean  $\pm$  S.E.)

Freezability group	Number of bulls	Free loose-heads (%)	Head abnormalities (%)	Acrosomal abnormalities (%)	Mid piece defects (%)	Tail defects (%)	Prox. cyto drop-lets (%)
I	9	0.88 $\pm$ 0.16	1.30 $\pm$ 0.25	1.48 $\pm$ 0.14	2.53 $\pm$ 0.37	1.35 $\pm$ 0.42	0.79 $\pm$ 0.24
II	4	0.98 $\pm$ 0.29	1.40 $\pm$ 0.34	1.92 $\pm$ 0.54	3.67 $\pm$ 0.88	1.03 $\pm$ 0.35	1.61 $\pm$ 0.94
III	7	0.80 $\pm$ 0.35	2.05 $\pm$ 0.29	1.93 $\pm$ 0.23	3.06 $\pm$ 0.53	1.17 $\pm$ 0.20	1.45 $\pm$ 0.26



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## Studies On Acrosomal Alterations In Deep Frozen Buffalo Spermatozoa

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### ABSTRACT

The acrosomal integrity of frozen buffalo spermatozoa during various stages of semen processing and post-thaw incubation was studied. The incidence of detached acrosomes increased from 8.8% in neat semen to 21.2% in incubated semen. The total incidence of other forms of acrosomal alterations such as swollen, ruffled, fractured and separating

acrosomes was 16.3% in neat semen, while it increased to 25.5% after equilibration, 44.5% after thawing and 47.5% after 3 hrs of incubation at 37°C. The pattern of changes of swollen and ruffled acrosomes was conspicuous in that, their incidence increased significantly after the end of equilibration and freezing respectively.

\* \* \*



Sperm acrosomal cap facilitates the entry of the sperm into the ovum and the fertilisation process may be impaired by abnormal acrosomes (Bedford *et al*, 1968). Besides the existence of a variety of acrosomal anomalies, alterations or injuries to acrosome occur during the different stages of semen processing and storage depending on the micro-environmental conditions around the sperm or the nature of sperm itself. Information on the nature and extent of acrosomal alterations in deep frozen buffalo spermatozoa is scarce. The present study, therefore, evaluates the effects of equilibration, freezing, thawing and incubation on acrosomal integrity of buffalo spermatozoa.

### Materials and Methods

Sixteen ejaculates from 4 fertile Murrah bulls obtained by artificial vagina were used in the study. Semen, after initial evaluation, was extended in Tris-egg yolk-glycerol (7% V/V) dilutor, equilibrated for 6 hrs and vapour frozen in medium French straws and then stored in liquid nitrogen for 24 hrs. Four different thawing procedures: 30°C for 30 s, 37°C for 15 s, 37°C for 30 s and 75°C for 9 s were used. Four straws per treatment within each ejaculate were pooled in test tubes and incubated in a water bath at 37°C for 3 hrs. The acrosomal alterations were studied by Giemsa staining technique and were classified as per Saacke *et al* (1968). A total of 200 spermatozoa per slide were examined under phase contrast microscope at 1000 $\times$  for estimation of percentage of different altered forms. Statistical analysis of the data were carried out after angular transformation of the percentages as per Snedecor and Cochran (1980).

### Results and Discussions

The results of semen evaluation for acrosomal integrity at different stages of semen processing and post-thaw incubation are presented in Table 1.

The acrosome membrane deterioration was more or less progressive at different stages of processing, the pattern of changes of swollen and ruffled acrosomes was conspicuous in that their incidence increased significantly ( $P < 0.01$ ) after the end of equilibration and freezing respectively (Table 1). Post-thaw incubation for 3 hrs had no effect on the pattern of acrosomal changes. The incidence of different forms of acrosomal alterations differed significantly between stages of processing of semen but not between different thawing procedures and bulls although bulls tended to vary in respect of swollen acrosomes (8.4 to 13.0%).

While similar studies are not available in buffaloes, Bandopadhyay *et al* (1983) reported higher incidence of detached acrosomes in fresh (23.6%) and thawed (47.1%) buffalo semen in citric acid whey dilutor which might reflect bull and dilutor differences. The different thawing temperatures used in the study (30°C to 75°C) are not associated with any deleterious effect on acrosomal integrity of buffalo spermatozoa. This agrees with the observation of Jainudeen and Dass (1982) who found improved acrosomal integrity by increasing the thawing temperature from 5°C to 50°C in Swamp buffaloes.

### Acknowledgements

The authors thank the Project Officer Indo Swiss Project, Visakhapatnam for providing the necessary facilities.



**Table 1: Incidence pattern of acrosomal changes of buffalo spermatozoa in Tris-egg yolk-glycerol diluter at different stages of processing and incubation.**

	Types of acrosomal changes					Total
	Detached	Swollen	Ruffled	Fractured	Separating	
Neat Semen	8.8 <sup>a</sup>	0 <sup>a</sup>	3.0 <sup>a</sup>	0 <sup>a</sup>	1.5 <sup>a</sup>	16.3 <sup>a</sup>
Equilibrated Semen	11.7 <sup>a</sup>	7.0 <sup>b</sup>	4.5 <sup>a</sup>	2.0 <sup>a</sup>	0.3 <sup>a</sup>	25.5 <sup>b</sup>
Thawed Semen	18.8 <sup>b</sup>	6.6 <sup>b</sup>	16.6 <sup>b</sup>	2.1 <sup>a</sup>	0.4 <sup>a</sup>	44.5 <sup>c</sup>
Incubated Semen	21.2 <sup>b</sup>	8.6 <sup>b</sup>	13.4 <sup>b</sup>	3.4 <sup>a</sup>	0.9 <sup>a</sup>	47.5 <sup>c</sup>
a, b = P > 0.05; a, c = P < 0.001						

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## Biometry Of Genitalia Of Exotic And Crossbred Bulls

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Clinical investigation and the studies on semen characteristics in bulls are important in estimating breeding efficiency. Testicular size and scrotal circumference have been significantly correlated with ejaculate volume and sperm concentration (Bora *et al*, 1982). Berry *et al* (1983) found a significant correlation of seminal vesicles with scrotal circumference and semen picture. The present communication reports the measurements of scrotal circumference, testicular size, volume and consistency and seminal vesicles in pure exotic and crossbred bulls.

\* \* \*

The study was conducted on 11 pure exotic (7 Holstein-Friesian, 4 Jersey), 5 crossbreds ( $\frac{1}{2}$ HF +  $\frac{1}{2}$ Sahiwal) and 7-triple crossbred with 75% exotic inheritance (4  $\frac{1}{2}$ HF +  $\frac{1}{4}$ BS +  $\frac{1}{4}$ Tharparkar, 3  $\frac{1}{2}$ HF +  $\frac{1}{4}$ Red Dane +  $\frac{1}{4}$ Sahiwal) bulls at the Dairy Farm, Punjab Agricultural University, Ludhiana and at Semen Bank, Patiala. The scrotal circumference of bulls was recorded by placing a flexible scrotal tape (Lane Manufacturing Inc., Denver, Colorado, U.S.A.) around its maximum diameter. The testicular length was measured with the help



of a divider by placing one arm of it on the proximal end and the other on the distal end of testis, excluding both caput and cauda epididymis. The maximum width of each testis was taken by placing one arm of the divider at the lateral aspect of the testis and the other at the point of its maximum width. The testicular thickness was recorded by placing one arm of the divider on the anterior aspect and the other on posterior aspect of the testis at the point of its maximum thickness. The divider was placed on a centimetric scale following each operation to record the measurements of length, width and thickness of testis. The volume of testes was calculated (Bedi, 1980). The testicular consistency was assessed with the help of a Tonometer (Lane Manufacturing Inc., Denver, Colorado, U.S.A.) at different places on each testis (Foote, 1978). The seminal vesicles were palpated per rectum for consistency and texture and their length and width were measured by pre-measured finger tips (Berry *et al.* 1983).

The recorded mean scrotal circumference in Holstein-Friesian bulls (Table 1) was in agreement with the findings of Mohanty (1981) and Coulter and Foote (1977, 1979). Scrotal circumference in KF breed (HF × Brown Swiss × Tharparkar) was in accordance with the findings of Hahn *et al.* (1969) in bulls of comparable age. The scrotal circumference in Jersey, HFS and HRS bulls was found to be lower. In all the genetic

groups studied the scrotal circumference was confirming to the standard suggested by Elmore *et al.* (1976) and was also corresponding to age for a normal breeding bull (Foote, 1978).

The measurements obtained for the length, width and thickness of testis in different breed group (Table 1) were in close agreement with that reported by Mohanty (1981) in HF bulls. The testicular measurements as well as testicular volume were found to vary in different breed groups under study. The mean testicular volume was highest in KF, followed by HFS breed and lowest in HRS bulls. Tonometer reading in the present study did not reveal any significant difference among the breed groups. The values were in general agreement with those of Rajkonwar (1979).

The mean seminal vesicular length and breadth (Table 1) of experimental bulls was found to be in agreement with that of Roberts (1971). The volume of seminal vesicle was recorded to be higher in HF ( $136.68 \pm 23.26 \text{ cm}^3$ ) and KF ( $129.25 \pm 41.85 \text{ cm}^3$ ) breeds, while in Jersey, HFS and HRS breeds, the volume of seminal vesicles was found to be below  $90 \text{ cm}^3$ . In the present study, the seminal vesicles as well as scrotal circumference in HF and KF bulls were recorded to be larger as compared to other bulls. Berry *et al.* (1983) also observed that larger seminal vesicles were associated with larger scrotal circumference.

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Table 1: Mean biometry of testes and seminal vesicles in different genetic groups of bulls (Mean  $\pm$  S.E.)

Genetic Group	No. of bulls	Scrotal circumference (cm)	Testicular measurements (cms)			Volume of testes (mm <sup>3</sup> )	Tonometer readings (mm)	Seminal vesicles (cm)	
			Length	Breadth	Thickness (mm <sup>3</sup> )			Length	Width
Holstein-Friesian (HF)	7	39.30 $\pm 0.69$	12.98 $\pm 0.36$	7.55 $\pm 0.19$	5.08 $\pm 0.08$	926.77 $\pm 21.66$	18.70 $\pm 0.28$	10.78 $\pm 0.41$	3.42 $\pm 0.23$
Jersey	4	35.67 $\pm 0.59$	12.93 $\pm 0.19$	7.45 $\pm 0.04$	5.30 $\pm 0.12$	1021.44 $\pm 13.39$	19.08 $\pm 0.27$	15.00 $\pm 0.00$	2.75 $\pm 0.17$
$1/2$ Holstein-Friesian + $1/2$ Sahiwal (HFS)	5	38.60 $\pm 0.56$	13.25 $\pm 0.35$	7.25 $\pm 0.17$	6.15 $\pm 0.22$	1183.45 $\pm 32.01$	19.69 $\pm 0.28$	9.55 $\pm 0.34$	2.60 $\pm 0.17$
$1/2$ Holstein-Friesian + $1/4$ Brown-Swiss + $1/4$ Tharparkar (KF)	4	39.87 $\pm 1.21$	13.37 $\pm 0.30$	7.81 $\pm 0.20$	6.90 $\pm 0.37$	1444.10 $\pm 46.35$	19.82 $\pm 0.11$	10.75 $\pm 0.63$	3.25 $\pm 0.30$
$1/2$ Holstein-Friesian + $1/4$ Red Dane + $1/4$ Sahiwal (HRS)	3	36.00 $\pm 0.27$	12.66 $\pm 0.09$	7.16 $\pm 0.09$	5.25 $\pm 0.08$	952.07 $\pm 10.51$	19.55 $\pm 0.19$	9.75 $\pm 0.11$	3.00 $\pm 0.00$



## Incidence Of Brucellosis In Breeding Bulls

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Bovine brucellosis is a disease of great economic importance and perhaps the most important zoonotic disease. Its occurrence in breeding bulls was reported by Rankin (1965), Plant *et al* (1976) and Deka *et al* (1982). The present investigation was undertaken to report the prevalence of brucellosis in breeding bulls of Assam.

Samples of serum and seminal plasma were collected from 56 breeding bulls maintained at different bull stations of ICDP in Assam. All these samples were subjected to standard tube agglutination test (Alton *et al*,

1975). The antigen used was procured from the Division of Biological Products, IVRI (U.P.). Out of 56 breeding bulls, 6 (10.7%) were positive reactors which indicated an alarming situation. Among the positive reactors, the antibody titre (IU/ml) varied from 80 to 160. This observation was lower than that (13.41%) of the earlier report of Deka *et al* (1982).

From this study, the importance of regular testing of breeding bulls for brucellosis and culling of positive reactors preferably every year is emphasized.

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## A Note On Rear Leg Conformation In Crossbred Breeding Bulls

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Perfect rear leg conformation is of vital importance for a breeding bull (Harrison *et al*, 1957 and Trimberger, 1977) to perform the service efficiently year after year. Information on rear leg conformation in breeding bulls is lacking. Only limited information is available in text books. Even though the defect may not interfere with breeding efficiency in young age, it is heritable and much more pronounced in the offspring and the production potential is reduced in the female progeny (Madan, 1983). Ott (1976) described some common structural faults of rear legs of bulls and

classified them as sickle hock, post legs, camped legs, bowed legs and toed out legs. In the present communication the incidence of faulty rear leg conformation in crossbred breeding bulls is reported.

Sixty-eight crossbred bulls stationed at All India Coordinated Research Project on Cattle, Lam Farm Guntur (A.P.) were critically examined to note the defective rear leg conformation. The defective rear leg conformations were classified as described by Ott (1976) and shown in Plate 1.



Plate 1

1. Desirable rear leg conformation from rear view.
2. Desirable rear leg conformation from side view.
3. Undesirable rear leg conformation toed out leg.
4. Undesirable rear leg conformation camped leg.
5. Undesirable rear leg conformation sickle hock.
6. Undesirable rear leg conformation post leg.

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Out of 68 cross bred bulls examined, the desirable and undesirable rear leg conformation was found in 43 (68.24%) and 25 (36.76%) bulls respectively. Different types of undesirable rear leg conformations observed in the present study were: Sickie hock in 3 (4.42%), post legs 7 (10.29%), camped legs 7 (10.29%) and toed out legs in 8 (11.76%) bulls. From this study it is evident that a considerable number of bulls did not have desirable rear leg conformation. This warrants a careful examination of rear leg

conformation of breeding bull before selection, as the defect is heritable.

It is concluded from the present observation, that the rear leg conformation of breeding bulls should be looked carefully before selection of a sire for regular breeding.

#### Acknowledgement

The authors are thankful to Senior Scientist, Cattle Project, Lam Farm, Guntur for the facilities provided to carryout this work.

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## Seminal Degeneration Due To Anaplasmosis In A Jersey Bull

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Jersey bull C-38, aged about  $5\frac{1}{2}$  years and weighing about 540 kg, belonging to the A.I. centre of the Department of Obstetrics and Gynaecology suffered a natural attack of anaplasmosis on 18.11.1976 and was under treatment till 8.12.1976. During the disease, the temperature of the animal ranged between  $100.8^{\circ}\text{F}$  to  $103.8^{\circ}\text{F}$ . The bull became weak and anaemic and no semen collection was possible. However, after the treatment was over, regular weekly collections were resumed.

#### Results and Discussions

The following parameters of semen were studied:

**Colour:** It became milky from the pre-disease creamy. This effect lasted for 6 weeks before becoming creamy again. Watson (1964) has reported these findings in rams.

**Density:** It became watery and returned to normal values 10-12 weeks post-treatment.

**Total ejaculate:** No significant change could be noticed. However Watson (1964) had



recorded a decrease in the total ejaculate in rams after tick fever.

*Initial motility:* During the post-treatment period up to 3 weeks, the initial motility was nil. During 4-6 weeks, it improved slightly and became 20%, 7-9 weeks it improved further and became 55% and finally from 10 weeks onwards it returned to the pre-disease level. These findings are in agreement with that of Watson (1964) in rams.

*pH:* No significant effect was observed.

*Sperm concentration:* It was significantly adversely affected till 9 weeks post-treatment and returned to normal during 10-12 weeks post-treatment.

*Live sperm percentage:* A significant decrease during 1-9 weeks post-treatment was recorded. It had not returned to normal till 10-12 weeks after treatment.

*Abnormal sperm percentage:* A significant increase in head, mid-piece and tail abnormality was recorded from 1-9, 1-12 and 4-9 weeks post-treatment period, respectively.

Among head abnormality detached, degenerated, tapering and pyriform heads; in middle-piece bent mid-piece and in tail bent tail, kinked tail and droplets abnormality (up to 27.33%) during 4-6 weeks, mid-piece abnormality (20.33%) upto 3 weeks and tail abnormality (23.33%) from 4-9 weeks post-treatment was recorded.

The present findings are in agreement with Watson (1964) who had reported upto 81% detached heads and 10% twisted tails in rams suffering from tick borne fever. Ratif *et al* (1971) had also reported morphological deviations in 2 bulls suffering from tick borne fever infection about 3-4 weeks after inoculation.

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## Effect Of Ascorbic Acid On Poor Sex Libido In Bulls

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Effect of ascorbic acid therapy on 3 breeding bulls having different degrees of sex-libido was studied. The animals were given 5 intramuscular injections of ascorbic acid on alternate days. The first 2 injections were of 1 gm each and the rest of 2 gm each. Sexual behaviour towards a cow in heat showed slight improvement. However the bulls returned to their pre-experiment level of sex libido, a week after the end of treatment.

Hultnas (1959) divided mating behaviour into: (a) libido defined as willingness and eagerness to mount and complete service. (b) Mating technique defined as the ability to perform a complete service.

Lagerlof *et al* (1956) reported that Indian bulls of milch breeds had poor serving ability, dual purpose breeds good, while draft breeds had very good serving ability. Philips (1940)



advocated 1 gm of ascorbic acid subcutaneously for improving breeding efficiency of bulls. Bortree *et al* (1942) observed that slow breeding bulls and hard to settle cows respond to ascorbic acid therapy.

#### Material and Methods

3 bulls, one each of Haryana, Sahiwal and Rath breed, belonging to the local A.I. centre were selected for this study. Their sexual behaviour towards a cow in heat was expressed as excellent (++++), good (+++), occasionally good (++) , poor (+) and nil, recorded daily during the treatment.

All the 3 bulls were given I.M. injection of 1 gm ascorbic acid dissolved in 5 ccs distilled water on alternate days. The treatment was repeated thrice per bull.

#### Results and Discussions

No appreciable change in the sexual behaviour of the bulls could be noticed after 2 injections (1 gm each) of ascorbic acid. However, improvement in sex libido was noticed after the bulls received 2 gm ascorbic acid each time. This was recorded as +++, ++ and + for the bulls which had ++, + and nil sex libido respectively at the start of treatment. It did not improve any further after 3 such injections and returned back to the pre-treatment stage, one week after the last injection.

The study showed that ascorbic acid stimulated sex libido to some extent only temporarily.

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## Dystocia Due To Foetal Muscular Hypertrophy In Buffaloes

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#### ABSTRACT

Two cases of foetal muscular hypertrophy leading to dystocia in pluriparous buffaloes have been reported. The method for their successful handling is also discussed.

\* \* \*

Dystocia in bovines can occur due to several factors which can be classified mainly into maternal and foetal causes. However, foeto-pelvic dis-proportion, due to relative narrowing of pelvis or due to increase in the size of foetus, may also be a factor responsible for dystocia. In anomalies or monstrosities in



the foetus, the malformed portions may obstruct the pelvis thereby causing dystocia (Arthur *et al*, 1982). The present report places on record the occurrence of dystocia in pluriparous buffaloes due to muscular hypertrophy in the calf.

**History and Examination:** Two pluriparous buffaloes with incomplete gestation (9 months and 8 months) were presented in the Veterinary Clinics of P.A.U., Ludhiana. History revealed that the expulsive efforts had started the previous day in both the animals with subsequent rupture of the water bags. Both the animals were active with usual feed and water intake. Temperature, pulse and respirations were within the normal range.

In case I, both the foetal hind limbs and one fore limb were taken out after amputating at the hip joints and the knee joint, by the local doctor without any success. P/V examination revealed the presence of one fore limb and the ribcage in the passage with the head lying deep inside the uterus.

In case II, on P/V examination, it was noticed that the cervix was not fully dilated. Both the forelimbs of the foetus were presented in the passage with left lateral deviation of the head.

**Obstetrical Management:** Manipulations in both the cases were carried out under the effect of epidural anaesthesia (2% Lignocaine Hydrochloride) and proper lubrication of the passage with parachlorgel. *Case I:* The neck was amputated from its base using Thygeson's foetotome. The thoracic and abdominal parts of the foetus were taken out by judicious traction applied to the presented secured fore limb. Lastly, the jammed doughy head and neck were removed by version and traction. *Case II:* Deviated head was corrected manually and brought into the passage. Traction applied to all the secured extremities (two for limbs and head) failed to deliver the

foetus. Although evisceration, following an incision above the xiphisternum, reduced the size of thorax and abdomen resulting in easy passage through the cervix, yet, the hind quarter got obstructed in the pelvis. Deep exploration revealed that the hind quarter was an oedematous muscular mass which was jammed in the pelvis. Foetus was bisected at the lumbar region and the cranial portion was removed. Pelvic bisection was necessitated to remove the limbs separately.

### Discussion

In bovines, the occurrence of dystocia due to local or generalised oedema of the foetus leading to increased size is rare (Arthur *et al*, 1982). In the present cases, there was increased muscular development in the foetal cervical region and hind quarter leading to abnormality in the contour, thereby causing obstruction in the normal passage of the foetus. In case I, there was diffused lumpy mass all around the cervical region extending upto the head. Dissection of the part revealed the presence of doughy oedematous muscular mass. Both the eyes were exophthalmic with parrot mouth (Fig. 1). Similarly in case II, the hind quarter was enlarged due to muscular hypertrophy which was confirmed

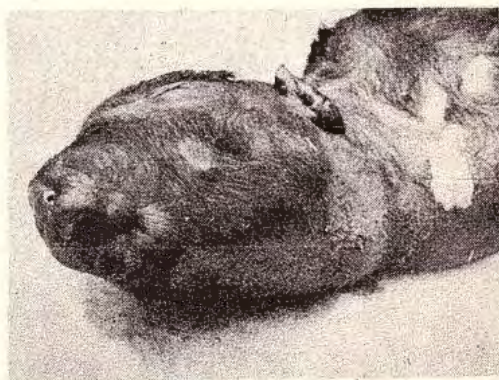


Fig. 1. Muscular hypertrophy of foetal neck.



histopathologically in both the cases. Sloss and Dufty (1980) have mentioned that excessive muscular development especially of hind quarter is of hereditary origin which may cause foeto-pelvic dis-proportion. The muscular development during early gestation

is by hyperplasia while during the later half, it is by hypertrophy (Prior and Laster, 1979). However, the exact reason for excessive muscular development specifically in the cervical region and the hind quarter in the present cases could not be pointed out.

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### Hydrallantois In A Goat — A Case Report

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Hydrallantois is common in most of the dropsical conditions affecting the foetus and its membranes. In cattle, this condition occurs mostly in twin pregnancies and is characterized by enormous abdominal enlargement. This condition has also been reported due to the presence of cystic kidneys or some defect in foetal renal tubules (Neal, 1956). In horses, it has been associated with foetal abnormalities (Vandeplasseche *et al*, 1976).

The present communication describes a case of Hydrallantois in a Barbari goat belonging to Institute livestock farm.

**Case Report:** An adult Barbari goat due for kidding for fourth time was brought with the history of abdominal enlargement (Fig. 1) on 9.3.88. This goat was bred on 15.11.87. For past one week, the animal was having mild

anorexia and discomfort in moving. There was difficulty in normal breathing for the last 2 days. The animal was under severe respiratory distress and unable to stand on the day of examination. On abdominal palpation conceptus could not be palpated. Abdomen was punctured on both sides by trocar and cannula to drain out the fluid. Although watery amber coloured fluid came out very slowly giving no relief to the animal. Seeing the severity of the case, it was immediately decided to perform caesarean section on 9.3.88.

**Surgical Procedure:** The site of operation at mid ventral side was shaved, cleaned and prepared for aseptic surgery. Thiopentone (Intraval Sodium) @ 400 mg was given intravenously for attaining anaesthesia and 15 ml of Xylocaine (2%) was used locally at

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Fig. 1. Barbari goat with distended abdomen

the site of incision. A 10 cm long incision was given and one uterine horn was brought to the incision site and a dorso-lateral incision was made in it. The watery fluid (Fig. 2) came out with pressure alongwith a dead male foetus. The fluid was allowed to drain as much as possible. Loose foetal membranes and other tissue debris were removed manually and two furea boluses were kept inside the horn and sutured. Now another uterine horn was incised to take out other foetus. A male dead kid and large amount of watery fluid was drained. The uterine horn was sutured after keeping two Furea boluses in it. The peritonium and muscular layers were sutured by chromic catgut. Skin wound was closed by interrupted sutures. Antibiotic coverage was followed for 7 days. The doe recovered perfectly and skin sutures were removed on 10th day.

Both the dead foetuses were examined for any type of abnormality. In both kids the kidneys were hypertrophied (Fig. 3) the details of which are as under:

Sr. No	Item	1st foetus	2nd foetus
i.	Weight (gms)	560.0	600.0
ii.	Weight of kidneys (gms)	8.60	8.75
iii.	Size of kidneys		
	Length cms	4.3	4.4
	Width cms	2.70	3.00
iv.	Vol. of fluid (discharges)	20 litres	

#### *Post Operative Reproductive Performance:*

The doe exhibited oestrus on 21.5.88 and was covered naturally. The animal was found pregnant on examination after 97 days of service by ultrasonic pregnancy detector. The animal kidded on 12.10.88 and delivered two kids (one male and one female). Male kid was however still born.

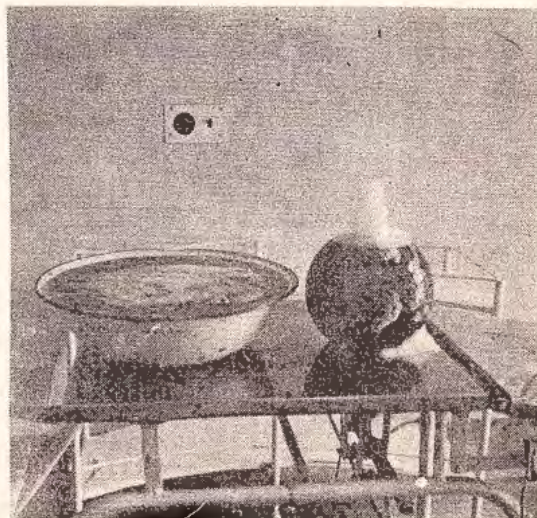


Fig. 2. Allantoic fluid collected during operation



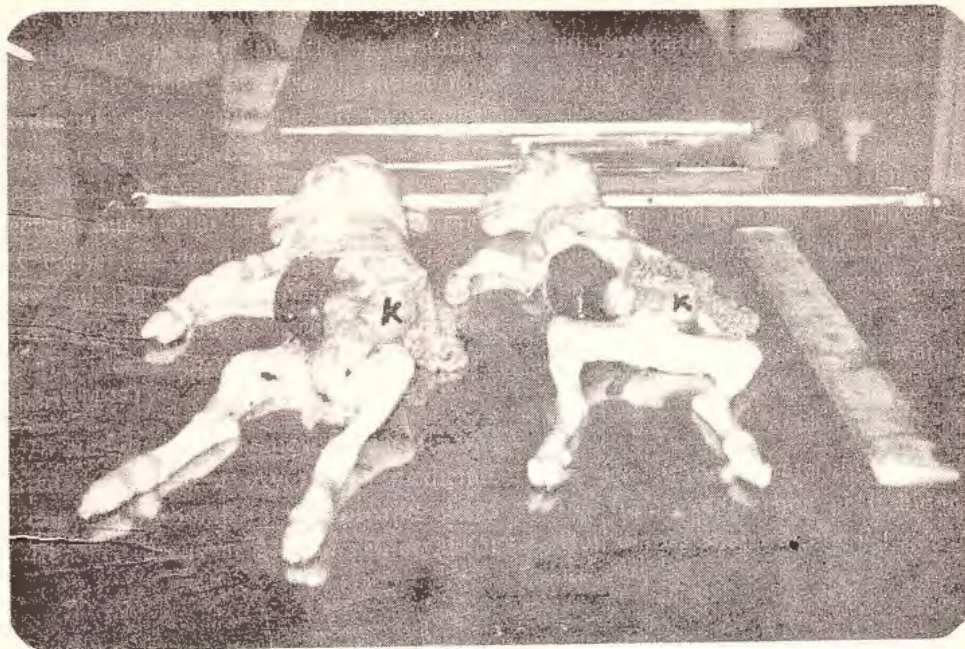


Fig. 3. Hypertrophic kidneys in both foetuses. K = Kidney

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### Herbogyne — The Uterine Ecboic For Macerated Foetus

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The Jersey heifer No. 148 of the Instructional Livestock Farm of the University, Khanapara Campus was of good health and conceived in the second puberal estrus. There was no udder development even after 6 months of pregnancy. She was examined per rectum and found that there was no definite integrity of the uterine horns,

cervix was hard, flattened and doughy cord like structure along the uterine body on the pelvic floor. The right ovary bore one regressing type of corpus luteum and the fremitus could not be felt. On squeezing the cervix, thick purulent discharge with necrotic smell could be noticed oozing out of the vulva. Hence the case was diagnosed to be macerated



foetus lodged inside the uterus. The heifer probably in the 4th month of gestation attempted to abort because of the serious nutritional stress, but due to incomplete dilatation of the cervix, the foetus could not be expelled out (Anderson, 1958). The serious effects of nutritional stress during pregnancy may cause this condition (Roberts, 1971; Sloss and Dufty, 1980). Maceration of the foetus took place due to ascending infection into the uterus through the dilated cervix Roberts (1971).

*Treatment:* After diagnosis, the heifer was treated with patent Ayurvedic medicine, Herbogyne (Herbolabs) at the dose rate of 225 ml drenched orally at 24 hours interval for

four consecutive days. After 20 hours of administration of the first dose, the heifer expelled out one 800 gm macerated foetus covered with macerated necrotic foetal membrane. Then the heifer was treated with Oxysteclin 30 ml (Sarabhai Chemicals, Baroda) intrauterine for three consecutive days to overcome the infections persisting in the uterus. The heifer resumed her normal estrus after 42 days of treatment and conceived to first insemination and gave birth to a normal female calf.

#### **Acknowledgement**

The authors gratefully acknowledge the free supply of 'Herbogyne' liquid inj. by M/s. Herbolabs, Shaharanpur Rd., Bereilly, U.P.

#### **REFERENCES**

- Andersen, L.G. (1958). Dystocia in the Bovine Animal. Vet. Rec. 70: 1175-1182.  
Roberts, S.J. (1971). In Veterinary Obstetrics and Genital Diseases. 2nd Edn. (Indian Ed.) Scientific Book Agency, Calcutta-1, PP. 126 and 174.  
Sloss, V. and Dufty, J.H. (1980). In Handbook of Bovine Obstetrics, Williams and Wilkins, Baltimore/London, PP. 113.

### **INDUCTION OF LIFE MEMBERS AN APPEAL**

The Office bearers of the ISSAR are striving their best to invigorate the association in all spheres of its professional activities. This is possible only if senior members take interest and play an active role in the affairs of the Association. The enrollment of Life Membership increased from 150 in 1986 to 290 in 1989, but there is a need for enlarging this membership. The response towards this effort has not been very encouraging. I, therefore, wish to appeal to all the members of our Association to strive their best to enroll additional Life Members. For further details, you may kindly contact our Treasurer, Dr. S.R. Pattabiraman, Professor of Clinics, Madras Veterinary College, Vepery, Madras — 600 007.

**A. RAMAMOHANA RAO**  
PRESIDENT — ISSAR



## From the Editor's Pen.....

Dear friends/Readers,

It has been a very trying but interesting experience to shoulder the arduous task of piloting IJAR from Vol. 8 (1987). You must have noticed that since last year (Vol. 9, 1988) our Journal is published punctually in due month. Brevity, clarity and punctuality have helped in increasing response which is evident from the No. of copies published.

We printed 600 copies of Vol. 8 (Nos. 1, 2) 1987, 800 copies of Vol. 9 (Nos. 1, 2) 1988 and now with the present issue Vol. 10 (No.1) 1989, 1200 copies are printed. We have tried to be as economic as possible Audited Statement of Accounts of IJAR Office at Nagpur upto date from 1-12-1986 to 31-3-1989 is published elsewhere in this issue. Perusal of the same will justify our statement. All this is inspite of the unpredictable escalation in the cost of paper, printing, postage and voluminous correspondence.

Right now due to Newsprint/paper shortage and high cost, many newspapers are closing down or have increased their price.

With the unstinted co-operation of you all, it has been possible to reach the present stage of IJAR. Kindly try to procure more financial support for your Journal by increasing Life Members, Institutional Subscriptions, Donations etc.

I also request the President and Executive Committee Members of ISSAR to give a serious thought for increasing the IJAR Subscription charges effective from Vol. 11 (1990) by taking a concrete decision in their next meeting.

With best wishes

NAGPUR  
May 31, 1989

Yours Sincerely,  
A.S. Kaikini  
EDITOR, IJAR

## From Secretary's Desk

Dear Member,

Through my appeals, circular letters, I have tried to focus your attention towards entries to be forwarded for consideration in respect of various awards instituted by our Society for the year 1988. These awards are Prof. Lagerlof Memorial Award, Dr. G.B. Singh Memorial Award, ISSAR Fellowship Award and recently introduced Best Chapter Award. I am expecting a sizeable number of entries from your respective States. Committees have been constituted for screening the entries for different awards and their decision will be binding on all of us.

I am happy to inform you that we have succeeded in procuring financial assistance from the Indian Council of Agricultural Research, New Delhi for holding National Symposium and also for publication of our Journal. Audited Statements of my office



for the year 1987 and 1988 are also published in this issue. The activities have been carried out economically.

I am further happy to indicate that the Gujarat State Chapter, the Vice-Chancellor, Gujarat Agriculture University, the Director of Animal Husbandry & Veterinary Service and various Dairy Co-operative societies from Gujarat State have come forward to host National Symposium on Animal Reproduction at Anand, some-time in November 1989. I hope all of you will be receiving announcement letter from the organisers confirming the dates of the symposium.

May I appeal to you all once again to try to enroll maximum number of annual subscribers, Life Members, Institutional Members or sustained members from your respective State and Secure/book maximum institutional subscribers and advertisements for our Journal (IJAR).

Yours Sincerely

D.R. PARGAONKAR  
Secretary, ISSAR

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#### DECLARATION

Statement about ownership and other particulars about THE INDIAN JOURNAL OF ANIMAL REPRODUCTION as required under Rule No. 8 of the Registration of Newspapers (Central) Rules, 1956.  
FORM No. IV (Rule No. 8)

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1. Place of Publication	Editorial Office : B-306, Ujwal Flats, Rahate Colony, Jail Road, Nagpur-440 022.
2. Periodicity of Publication	Bi-annual (June & December).
3. Printer's Name	Majestic Printing Press.
Nationality	Indian
Address	Near Tilak Statuc, Mahal, Nagpur-440 002
4. Publisher's Name	Dr. D. R. Pargaonkar, Secretary, ISSAR.
Nationality	Indian
Address	Parbhani Veterinary College, Parbhani-431 402.
5. Editor's Name	Dr A. S. Kaikini, Ex-Dean. Faculty of Veterinary Science, PKV, Akola.
Nationality	Indian
Address	B-306, Ujwal Flats, Rahate Colony, Jail Road, Nagpur-440 022.
Names and addresses of individuals who own the newspaper and partners, share holders holding more than 1 per cent of the total Capital	Official Organ of THE INDIAN SOCIETY FOR THE STUDY OF ANIMAL REPRODUCTION (ISSAR) Regd. No. Bom. 253/78. Office : Department of Gynaecology & Animal Reproduction, MAU, Parbhani-431 402.

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I, Dr. A. S. Kaikini, 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.

Dr. A. S. Kaikini

Editor, The Indian Journal of Animal Reproduction  
The Indian Society for the Study of Animal Reproduction

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**Receipt and Payment Statement of ISSAR Secretary's Office  
For the Year Ended, 1987**

RECEIPTS		PAYMENTS	
To,	Rs. Ps.	By,	Rs. Ps.
Op. Balance	Nil	Postage and Telegram	282-50*
Received from Treasurer, ISSAR,		Printing Charges	180-00
Madras by D.D.	500-00	Block Charges	40-00
Hand Loan from		Stationery, Envelops	22-00
Dr. Pargoankar Sec. ISSAR	400-00	Travelling	136-00
		Xeroxing	239-50
		Closing Balance	Nil
	<u>900-00</u>		<u>900-00</u>

* Telegram Charges	16-50
Regd. Post Parcel	74-00
Postage Stamps	192-00
	<u>282-50</u>

**For the Year Ended, 1988**

RECEIPTS		PAYMENTS	
To,	Rs. Ps.	By,	Rs. Ps.
Op. Balance	Nil	Postage & Telegram	1,116-50*
Received from the treasurer,		Printing	1,210-00
ISSAR Madras by D.D.	4,000-00	Silver Medals	1,106-00
Hand Loan from Dr. Pargaonkar,		Cash Prizes	202-00
Secretary ISSAR	602-00	Stationery	24-00
		Travelling	146-00
		Xeroxing	154-00
		Miscellaneous	243-50
		Hand Loan repaid to the	
		Secretary ISSAR Dr. Pargaonkar	400-00
		Closing Balance	Nil
	<u>4,602-00</u>		<u>4,602-00</u>

Examined and found correct as per the books of accounts and vouchers produced.

* Telegram Charges	108-00
Regd. Post Parcel	764-90
Postage Stamps	243-60
	<u>1,116-50</u>

Parbhani,  
Dated : 17-4-1989

**T. M. SHAH M. No. 35396**  
CHARTERED ACCOUNTANTS  
Peda Hanuman, Station Road  
Parbhani-431 401



**INDIAN JOURNAL OF ANIMAL REPRODUCTION**  
**Receipts and Payments Account for the Period 1-12-1986 to 31-3-1989**

RECEIPTS		PAYMENTS	
To,	Rs. Ps.	By,	Rs. Ps.
Receipts from Treasurer (ISSAR)	41,000-00	Journal Printing	49,564-50
Subscription (Institutions)	18,374-00	(Vol. 8- June 87 & Dec. 87	
Advertisement	21,555-00	600 copies each)	
Block Making	4,525-00	(Vol. 9- June 88 & Dec. 88	
Interest on Savings Bank A/C	1,029-35	800 copies each)	
		Office Maintenance Expenses	1,207-00
		Postage	4,896-10
		Bank Commission	268-00
		Stationery and Printing	3,680-25
		Conveyance	1,355-00
		Packing Charges of Journal	335-80
		<b>Closing Balance :</b>	
		Cash in Hand	345-85
		Cash with State	
		Bank of India	
		A/C No. 61294	24,830-85
			25,176-70
<b>Total</b>	<u>86,483-35</u>	<b>Total</b>	<u>86,483-35</u>

Certified that the figures shown in the above Receipts and Payments Account of "Indian Journal of Animal Reproduction" for the period 1-12-1986 to 31-3-1989 agree with the books and vouchers maintained which have been audited by us and are found to be correct.

Nagpur  
Dated : 14-4-1989

**C. R. SAGDEO & Co.**  
**CHARTERED ACCOUNTANTS**  
Prabha Niwas, Jail Road,  
Nagpur-440 022



## INDIAN JOURNAL OF ANIMAL REPRODUCTION

### GUIDE LINES TO AUTHORS

1. The Journal is published twice a year as a Volume comprising of June and December issues.
2. Paper should be TYPE-WRITTEN and double spaced all throughout (including references and tables) on white, durable bond paper of size 22 cm × 28 cm, with a 4 cm margin at the top, bottom and left hand side. Articles including illustrations, should be sent in duplicate after a careful check-up of typographical errors.
3. Articles should not exceed Six typed pages. Short Communications/Research notes and clinical articles should be limited to two typed pages.
4. A recent issue of the Journal be consulted for the format of articles and methods of citation of references in the text as well as at the end of the article.
5. The Abstract and Introduction should be brief. Review of Literature should be crisp and pertinent to the problem. The main emphasis in the text should be on the actual work done by the author(s). Details of Materials and Methods including experimental design and techniques used should be given. Where the methods are well known, the citation of standard work is sufficient. References should be reduced to the barest minimum.
6. Mean results with the relevant standard errors should be presented rather than detailed data. The statistical methods used should be clearly stated. Tables should be minimum and fit in the normal layout of the page. All weights and measures should be in Metric units.
7. The Results and Discussion should be combined to avoid repetition. The Discussion should relate to the limitations or advantages of the author's actual work in comparison with that of others.
8. All articles are sent to Referees for scrutiny and author(s) should meet criticism by suitably revising, the article. Block-making charges of Photographs, Tables, Graphs, Histograms and Line drawings appearing in the accepted articles shall have to be paid by the concerned author(s) in advance on receipt of the Bill from the Editor, I.J.A.R.
9. All efforts are made to acknowledge, process and accept the articles received for early publication. Authors are earnestly requested to become active paying subscribers of the Journal and help procure/book suitable advertisements for publication in the Journal to strengthen its financial resources.
10. Articles and all matters pertaining to the Journal be sent to the Editor, Indian Journal of Animal Reproduction, B-306, Ujwal Flats, Rahate Colony, Jail Road, Nagpur-440 022.



# ISSAR NEWS

## Indian Veterinary Association Prof. Nils Lagerlof Memorial Prize

We are happy to announce that Prof. Nils Lagerlof Memorial Medals are awarded jointly to:

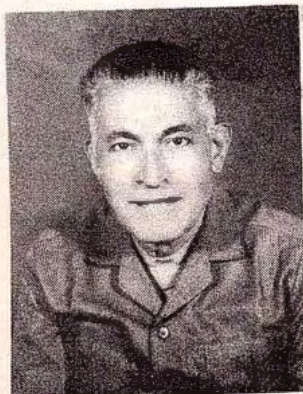
1983

Drs. C.V. Dindorkar, A.S. Kaikini and A.R. Sheth, Department of Post Graduate studies in Gynaecology and Animal Reproduction Post Graduate Institute, Punjabrao Krishi Vidyapeeth, Akola for their article titled "FSH Blood Serum level in Red Dane, SRB and Jersey cross-breds during various phases of reproduction"—Indian Vet. J. 60(9):727-730.

Dr. C.V. Dindorkar, Ph.D. is Associate Professor of Gynaecology, Nagpur Veterinary College. Earlier, he was awarded Gold Medal of Udaipur University for standing first at the MVSc Examination, 1969.



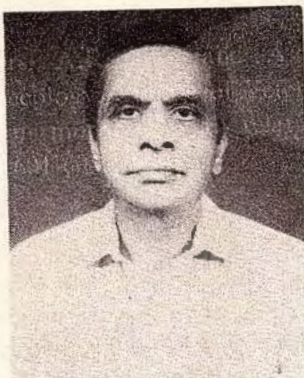
Dr. C.V. Dindorkar



Dr. A.S. Kaikini

Dr. A.S. Kaikini, Ph.D FRVCS (Sweden) Fellow ISSAR, is Emeritus Scientist (ICAR) and Ex-Dean, Faculty of Veterinary Science, PKV, Akola; Head, Deptt. of Gynaecology to Surgery PKV & Associate Dean, Nagpur Veterinary College. Presently Editor, Indian Journal of Animal Reproduction. Best University Teacher of PKV for Gold Medal Award of ICAR Golden Jubilee (1982).





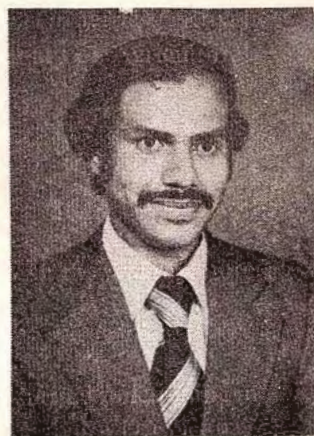
Dr. A.R. Sheth

Dr. A.R. Sheth, Ph.D. is Director Grade Scientist in-charge Institute of Research in Reproduction (ICMR), Parel, Bombay-12

1984

Dr. N. Baishya, K.K. Saharia and C.K. Rajkonwar, Department of Obstetrics, Gynaecology & A.I. Faculty of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati for their article titled "Therapeutic use of Prostaglandin  $F_{2\alpha}$  in the treatment of bovine pyometra". — Indian Vet. J. 61(3):246-249.

Dr. N. Baishya is Associate Professor of Gynaecology, Assam Veterinary College, Guwahati. Earlier, he was a recipient of the coveted George Fleming Prize awarded by British Veterinary Association for best clinical article in 1981.



Dr. N. Baishya



Dr. C.K. Rajkonwar

Dr. C.K. Rajkonwar, Ph.D. is the Associated Dean, Lakhimpur College of Veterinary Science, A.A.U., North Lakhimpur (Assam) and Vice-President, ISSAR.



Drs. A.R. Rao and A. Bane, Department of Animal Reproduction & Gynaecology, College of Veterinary Science, Tirupati (A.P.), for their article titled "Sperm morphology in relation to age in normal fertile bulls"—Indian Vet. J 62(7):601-604.

Dr. A. Ramamohana Rao, Ph.D. FRVCS (Sweden), Fellow ISSAR is Dean, Post Graduate Studies, APAU, Hyderabad and President, ISSAR.



Dr. A. Bane



Dr. A.R. Rao

Dr. Allan Bane, DVM is Professor Emeritus, Department of Gynaecology & Obstetrics, Royal Veterinary College, Swedish University of Agricultural Sciences, Uppsala (Sweden).

We heartily greet and congratulate all these awardees and wish them many more laurels in future in their field of specialisation.

\* \* \*

We are glad to inform that the Bihar Chapter of ISSAR has started functioning in right earnest from April 1989 under the leadership of Dr. B.K. Singh, Ph.D. (Vienna), University Professor & Head Department of Gynaecology, Ranchi Veterinary College Ranchi. Dr. Balraj Singh, Asstt. Professor of Gynaecology, Ranchi is the Hon. Treasurer of this Chapter.

\* \* \*

We are glad to inform that Dr. R.C. Gupta, Professor and Head, Department of Gynaecology & Obstetrics, College of Veterinary Science, HAU, Hisar is appointed Dean, Faculty of Veterinary Science, Haryana Agricultural University, Hisar. Under his talented, experienced and dynamic leadership, the faculty has a bright future.

We congratulate him and wish him all success.

\* \* \*



Dr. S.K. Verma, Ph.D. is promoted and appointed Professor and Head, Department of Gynaecology & Obstetrics, College of Veterinary Science, Haryana Agricultural University, Hissar.

We congratulate Dr. Verma and wish him best of luck.

\* \* \*

Dr. Ingemar Settergren, FAO/SIDA Expert in Animal Reproduction, was in India from 1st to 4th March 1989, during which he visited Madras Veterinary College to discuss about the progress of research and education in the field of Animal Reproduction.

\* \* \*

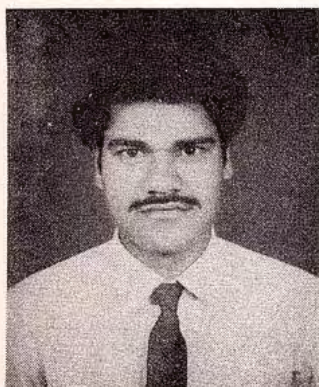
We are happy to learn that Dr. C. Krishna Rao, GMVC, BVSc, M.S., Ph.D. Fellow ISSAR, Ex-Animal Husbandry Commissioner with the Government of India, Ex-Vice-Chancellor, Andhra Pradesh Agricultural University and Ex-President, Indian Veterinary Association was honoured by the Chandra Shekhar Azad University of Agriculture and Technology Kanpur (U.P.) with the Degree of "Doctor of Science (Honoris Causa)" at its convocation held on 11th February 1989.

We congratulate Dr. C.K. Rao for the distinction conferred on him and wish him a healthy long life in the service of Veterinary & Animal Sciences in India.



Dr. C. Krishna Rao

\* \* \*



Dr. D.M. Mondhe

Dr. D.M. Mondhe, MVSc, ISSAR LM-942, Livestock Development Officer, Mittewani (Maharashtra) is selected as Trainee, Department of Biotechnology, Govt. of India. Dr. Mondhe had a distinguished academic career standing First in Gynaecology & obstetrics in the ICAR Junior Research Fellowship Examination, 1985.

We wish this talented young scientist more laurels and best luck in future.

\* \* \*