Vol. 11 No. 2
DECEMBER, 1990

ISSN 0970-2997

114

The Indian Journal of Animal Reproduction

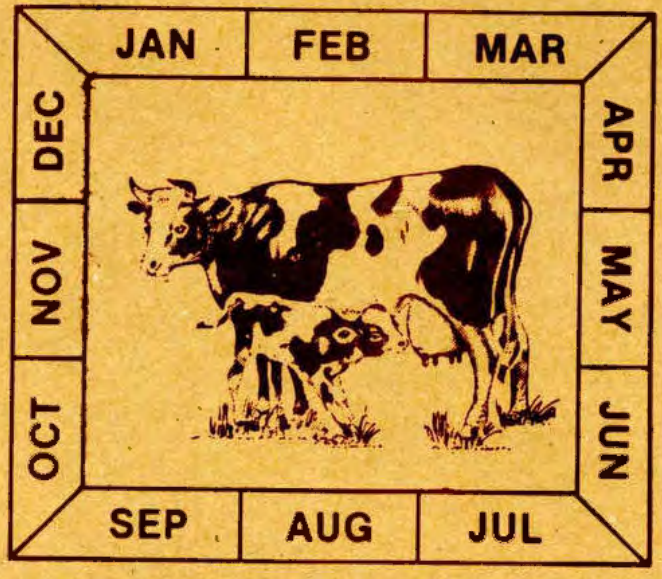
JOURNAL OF THE
INDIAN SOCIETY FOR THE
STUDY OF
ANIMAL REPRODUCTION

(Regd. No. Bom. 253/78)

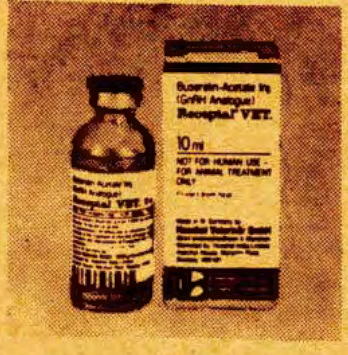
Receptal®

HIGHLY POTENT AND SAFE 'GnRH' ANALOGUE
FOR TREATMENT OF OVARIAN DISORERS IN
COWS MARES & RABBITS

A MODERN TOOL FOR



CALF A YEAR PROGRAMME



INDICATION

- DELAYED OVULATION, ANOVULATION
- ANOESTRUS
- FOLLICULAR CYST
- IMPROVEMENT OF CONCEPTION RATE AFTER ARTIFICIAL INSEMINATION AND OESTRUS SYNCHRONIZATION
- PROPHYLAXIS OF FERTIUTY DISORDERS BY EARLY INDUCTION OF CYCLE

PRESENTATION

10 ml. vial

Hoechst India Limited
Hoechst House, Nariman Point, Bombay-400 021.

Hoechst

ISSN 0970-2997

THE INDIAN JOURNAL OF ANIMAL REPRODUCTION

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

(Regd. No. Bom. 253/78)

Vol. 11 **No. 2** **December 1990**

Annual Subscription Payable in Advance :—

Rs. 100/— to all Public/Government and University Institutions/Libraries. Foreign U.S. $ 100 by Air. Free to Life Members/Regular Members/Student Members

The Indian Society for the Study of Animal Reproduction
(Regd. No. Bom. 253/78)

EDITORIAL

On reviewing the Veterinary and Animal Sciences progress in post-independance era, we find that vast all round advances in these fields have been achieved in India and a time has come when they be given a status equal to and on par with Agricultural Sciences at all levels including teaching, research and extension education. This is a dire National necessity to accelerate progress in these Sciences, the ultimate fruits of which are rural development and economic benefit to the Farmers, Cattle breeders, Dairy enterpreuners, landless labourers and people below the poverty line or BPL population.

Realising its urgency, an independant University of Veterinary and Animal Sciences has been started from 1988 at Madras in Tamilnadu State, which is a super positive step in the right direction. These Sciences cannot develope with an "also ran" secondary status as a bandwagon to the plethora of crop sciences in an Agricultural University. Tamilnadu State deserves all compliments for breaking the ice. We learn that Gujarat State is also starting a separate University of Veterinary Sciences and Animal Husbandry. Best luck to them.

This process should be accelerated in all the Major States of India and be completed in this decade for speedy implementation and heralding our onward march in the 21st Century. We appeal to all concerned to give a serious thought to this vital aspect and strive for its achievement. Tamilnadu has shown the way and let us follow it at the State and National levels. A co-ordinated and concentrated effort in this direction is essential.

* * *

We thank all our Life Members, readers, well-wishers referees, contributors, subscribers and advertisers for their keen and ready co-operation extended to IJAR and wish them a very Happy and Prosperous New Year-1991 and crave their sustained indulgence in future.

— EDITORIAL BOARD

ISSN 0970-2997

THE INDIAN JOURNAL OF ANIMAL REPRODUCTION

CONTENTS

Vol. 11 No. 2 December 1990

1. Gynaecology & Obstetrics

THE INDIAN JOURNAL OF ANIMAL REPRODUCTION

ATTENTION

All Authors/Contributors should note that :—

1. Articles should be in accordance with the style of the Journal and in confirmity to the Guide Lines to Authors published in IJAR.

2. Table composing charges, Block making charges and cost of Reprints shall have to be paid by the Authors as per Bills sent to them. Responsibility of settlement of such Bills shall be of the Author/ Head of Department who sent the article to IJAR or the Author indicated by the forwarding authority.

3. Authors are requested to pay the Bills promptly without entering into avoidable correspondence. Kindly clear pending Bills without any further delay.

4. Decision of the Referees shall be binding on the author(s). No correspondence in this matter will be entertained.

5. Please note that IJAR is printed by Offset process. Advertisement matter positive print or its negative only need be enclosed with advertisement order. Blocks need not be sent.

Help your IJAR to serve you better !

20-11-90	**D. R. Pargaonkar** Secretary, ISSAR	**A. S. Kalkini** Editor, IJAR	**A. R. Rao** President, ISSAR

DR. ASIM BALA, M.V.Sc., PH.D.
MEMBER OF PARLIAMENT
(LOK SABHA)

109, North Avenue,
New Delhi.

Dt.12.9.90

Dear Dr. Kaikini,

I am very glad to receive your fine letter which given me much pleasure. Sorry for my delay reply but I am always fast with the profession.

As per your request I am sending my biodata and a photograph for your journal. I think your journal, i.e. Indian Journal of Animal Reproduction, will explore the development of livestock production in the country specially rural economy. You will be happy to know that the Government of India is exporting animal products ten percent of the total export. It would be double if we organised this in a befitting manner. You kindly convey my heartiest thank to all of your members. Good bye.

With regards,

Yours sincerely,

Asim Bala
(ASIM BALA)

Dr. A.S. Kaikini,
B-306 Ujwal Flats,
Rahate Colony, Jail Road,
NAGPUR:440022

NATIONAL SYMPOSIUM ON

RECENT BIOTECHNOLOGICAL ADVANCES IN ANIMAL REPRODUCTION

AND IX ANNUAL CONVENTION OF

The Indian Society for the Study of Animal Reproduction

Organised by :

I.S.S.A.R; H.A.U., HISAR AND I.C.A.R.

at

HISAR, FEB 6-8, 1991

Telephone : 01662-3721 Extn 4213
Telegram : AGRIVARSITY

Organising Secretary :
Dr. S. K. Khar *Prof. & Head*
Deptt. of Gynae. & Obsts.
College of Vety. Sciences
H.A.U., Hisar

Scientists participating in the Symposium are requested to ensure that their Registration Forms/ Delegation Fees are received by the Organising Secretary by December 31, 1990.

Organising Secretary
National Symposium

IJAR:11:2:79-84:1990

Studies On Semen Freezability Of Pure Jersey And Crossbred Bulls With Varying Levels Of Exotic Inheritance.

L.R. SAGDEO[1], A.B. CHITNIS[2], S.N. DESHMUKH[3], and A.S. KAIKINI[4].

Frozen Semen Laboratory, A.I. Centre, Telankhedy, Nagpur-440 001.

ABSTRACT

Freezability in 80 breeding bulls comprising of 14 pure Jersey (Jy), six 75% Jy, nine 62.5% Jy, twenty nine 50% Jy, eight 75% Holstein Friesian (HF) and fourteen 62.5% genous inheritance (12.5 to 50%) of Gaolao, Tharparkar, Hariana and Gir breeds. Freezability was 100% in case of Jy pure breds but rejection of bulls was highest in case of 75% HF (87.5%), followed by 75% Jy (83.34%), 62.5% HF (78.58%), 50% Jy (75.87%), and lowest (44.5%) in 62.5% Jy crossbred bulls. The post-thaw motility was highest in Jy Pure breds (47.14 ± 0.92%) followed by 62.5% Jy (44.16 ± 0.99%) and 50% Jy (42.65 ± 0.99%), whereas 75% Jy and 62.5% HF showed similar values (28.33%). 75% HF recorded lowest value (23.64 ± 5.32%) C.V. (77.5 and 64.95%) indicated low freezability in both 75% Jy and HF cross bred bulls. 62.5% Jy crosses proved better (33.15%) over HF crosses (51.75%), whereas 50% Jy crosses had least variation (25.32%) indicating better freezability. These studies indicate that Exotic: indigenous combinations play a major role in cross bred bull semen freezability. Further cytogenetic studies are essential to solve this enigma.

* * *

Frozen Semen technology is extensively used in India for ushering in white revolution by increasing milk productivity of Livestock through cross breeding by A.I. The cross breeding policy requires inter-se maintenance of exotic inheritance in the subsequent generations of cross breds ranging from 50 to 62.5%, which indicates the imperative use of crossbred bulls for such future breeding programmes. According to Mathew (1981), the semen ejaculates of all bulls are not equally freezable. Scanty data pertaining to freezability of semen in crossbred bulls is available, even though it is a major problem in them as compared to pure bred exotic or indigenous bulls or buffalo bulls. The present comparative study on freezability of crossbred bulls vis-a-vis pure exotic bulls are therefore undertaken.

Material and Methods

These studies were carried out during a 2 1/2 year period (Nov. 85 to May, 88) on 80 selected bulls located at Frozen Semen station, Nagpur. The breeding bulls comprised of 14 pure Jersey (Jy), six 75% Jy, nine 62.5% Jy, twenty nine 50% Jy, eight 75% Holstein Friesian (HF) and fourteen HF 62.5%. In all six genetic groups were studied.

1. Asstt. Director of Animal Husbandry, District A.I. Centre, Wardha-442001.

2. Asstt. Director of Animal Husbandry, (Frozen Semen). Nagpur-440001.

3. Associate Professor of Genetics and Breeding, Nagpur Veterinary College, Nagpur-440006.

4. Ex-Dean, Faculty of Veterinary Science PKV and Emeritus Scientist (ICAR), Nagpur Veterinary College, Nagpur-440006.

Range of age in case of Jy pure bred bulls was 16 to 98 months and in case of all other bred bulls it was 18 to 70 months. In cross bred bulls the indigenous inheritance included various levels (12.5 to 50%) of Tharparkar, Gaolao, Hariana and Gir breeds. All the bulls were in good health, free from any disorder of genital tract and maintained under identical managemental practices.

Semen ejaculates were collected by AV technique twice a week. Semen samples having Density DD and above, mass activity ++(+) and above and initial motility 70% and above were subjected to freezability. Tris egg yolk glycerol (6%) dilutor was used. Antibiotics were added to the dilutor @1 mg/ml dilutor. Freezing was carried out in 0.25 straws (Land Shut Method). Four hours equilibration time was given for semen straws at 5^0C temperature. Horizontal vapour freezing technique was used (Mathew *et.al.*1982). Post-thaw (P.T.) motility was observed 30 minutes after freezing. Thawing was carried out in 37^0C water for 15 seconds. Frozen semen samples having 40% motility (P.T.) and above were considered as freezable. The samples showing less than 35% P.T. Motility were discarded as per Mathew (1974). The relevant data was statistically analysed as per Snedecor and Cochran (1967).

Results

Mean, Range, Standard Error and Coefficient of Variation for Volume (ml), mass activity (+ 0 to 5 scale), initial and Post thaw (P.T.) motility (%) of bulls belonging to six genetic groups under study are recorded in Table 1. The Analysis of variance is presented in Table 2.

(1) *Volume*: The results revealed the maximum semen volume of 3.99 ± 0.18 ml. in 75% HF (HF x Gir) and lowest (2.23 ± 0.18 ml) in 75% Jy crossbreds. The coefficient of variability (C.V.) ranged from 41.22 to 55.16% among six genetic groups studied, indicating great degree of variation in volume of semen. In 75% HF crosses, slightly lower values were recorded (3.2 to 3.5 ml) by Bakshi (1980). Sharma *et.al.* (1986) in their study of seminal characters recorded slightly more semen values (4.6 ± 0.4 ml) in 11 crossbred bulls (B.S. x HF x Gir).

In 50% Jy crossbreds, these values were 3.24 ± 0.11 ml. Roy *et.al.* (1975) recorded slightly higher values (4.1 ± 0.31 ml) of volume in five Jy x Hariana 50% bulls of 21 to 28 months age group. But Nair and Varadrajan (1985) recorded slightly less values (2.07 ml) in 50% Brown Swiss bulls.

In 62.5% HF, 3.10 ± 0.15 ml volume of semen was recorded. Similar values were recorded by Raja and Rao (1983) in case of 62.5% Brown Swiss bulls ranging from 1.5 to 3.5 ml. and slightly higher bulls, values in 62.5% Jy (3.73 ± 0.24 ml).

Mean values for semen ejaculates in case of Jersey pure bred bulls were 2.79 ± 0.15 ml. This value is quite lower than the value (3.92 to 5.25 ml) reported by Kohali (1980).

Further, critical difference (C.D.) test indicated that the average semen volume was significantly lowest for 75% Jy bulls. Average semen volume of Jy pure bred bulls was also significantly lower than other genetic groups except 75% Jy bulls in which it was lowest (2.23 ± 0.18 ml).

The average semen volume of 50% Jy and 62.5% HF bulls was similar, but slightly lower than 62.5% Jy and 75% HF bulls. The average

value for 62.5% Jy bulls was significantly higher (3.73 ± 0.24 ml.) than other genetic groups except 75% HF bulls which showed significantly highest average semen volume (3.99 ± 0.24 ml).

(2) *Mass Activity* : Average mass activity recorded in this study was 3.04 ± 0.03, 2.07 ± 0.12, 2.30 ± 0.15, 1.93 ± 0.06, 1.77 ± 0.19 and 2.27 ± 0.08 for pure Jy, 75% Jy, 62.5% Jy, 50% Jy, 75% HF and 62.5% HF respectively. These values were lower in Pure bred Jy bulls (3.04 ± 0.03) than those reported by Kohali (1980) which ranged from 3.68 to 4.26. In case of 75% Jy bulls, values recorded in this study (2.07 ± 0.12) were lower than those recorded (3.48 ± 0.17 and 3.8 ± 0.1) by Baburao and Rao (1990) and bySharma *et.al.* (1986) respectively.

For 50% Jy bulls value recorded was 1.93 ± 0.06 which is quite lower than that (3.62 ± 0.14) reported by Tuli *et.al.* (1988). However Tomar *et.al.* (1985) recorded comparable values of 2.04 ± 0.2 and 2.36 ± 0.14 in 25 to 30 months and 31 to 36 months old, HF x Hariana crossbred bulls.

There was least variation in mass activity (6.05%) of semen in case of Jy pure bred bulls while there was great degree of variation (31.88 to 48.26%) amongst the other five genetic groups. Highest value for mass activity (3.04 ± 0.03%) was observed in Jy pure bred bulls and lowest (1.77 ± 0.01) in 75% HF bulls.

50% and 75% Jy bulls showed similar values. 62.5% Jy and HF bulls were also similar in this respect but were slightly superior to 75% HF bulls.

(3) *Initial Motility* : Highest Mean initial Motility of 72.14 ± 0.65% was recorded for semen samples of Jy pure bred bulls, while lowest (30.24 ± 2.94%) was recorded in case of 75% HF bulls. Corresponding values for the other genetic groups were 75% Jy (47.50 ± 4.62%), 62.5% Jy (52.07 ± 1.77), and 62.5% HF (46.69 ± 2.23%)

C.V. indicated lowest variation (6.80%) in initial motility of Jy Pure bred bulls, whereas the other five genetic groups exhibited greater variation of 48.97 to 74.46%

Mathew *et.al.* (1982) recorded higher mean values of 63.98 and 67.5% in case of 75% Brown Swiss bulls between age groups of 2 to 4 and 4 to 6 yrs respectively. Chauhan *et.al.* (1983) also recorded higher motility of 65.77 ± 3% in 75% cross bred bulls which is much higher than that in 75% HF (30.24 ± 1.77%) and 75% Jy crossbred bulls (47.50 ± 4.62%) recorded in this study. Mean values in case of 62.5%. Brown swiss bulls between age groups of 2 to 4 and 4 to 6 yrs. were also higher (64.38 and 65.06%) than 62.5% Jy (52.07 ± 3.49) and 65.5% HF (46.69 ± 2.23%) recorded in this study. Values for Jy pure bred bulls in this study were higher (72.14 ± 0.65%) than values recorded by Mathew *et.al.* (1982) for pure Brown Swiss bulls between age group of 2 to 4 (64.38%) and 4 to 6 (65.06%) years.

In 50% Jy crossbred bulls initial motility was 37.24 ± 1.77% which is much lower than that recorded (63.77 ± 2.48%) by Tuli *et.al.* (1988) in Jy x Haryana, HF x Haryana and Brown Swiss x Haryana crossbred bulls.

(4) *Post Thaw Motility. (P.T.M.)*: The mean P.T.M. recorded for six genetic groups in this study was 47.14 ± 0.92, 28.33 ± 5.26%, 44.16 ± 2.99%, 42.65 ± 1.54%, 23.64 ± 5.32% and 28.33 ± 2.35% for pure Jy, 75% Jy, 62.5% Jy, 50% Jy, 75% HF and 62.5% HF bulls respectively.

The higher P.T.M. (47.14 ± 0.92%) recorded for Jy Pure bred bulls in this study is comparable to overall mean P.T.M. (46.73%) recorded in Pure Brown Swiss bulls by Mathew *et.al.* (1982).

In case of 75% Jy crossbred bulls lowest P.T.M. of 28.33 ± 5.26%, was recorded in this study, which is lower than that (49.39%) in 75% Brown Swiss bulls recorded by Mathew *et.al.* (1982).

The mean value (46.16 ± 2.99%) of PTM recorded in 62.5% Jy cross bred bulls is comparable with the mean values (44.79%) recorded by Mathew *et.al.* (1982).

The mean value (42.65 ± 1.54%) of PTM obtained in 50% Jy bulls is similar to that recorded (43.33 ± 1.86%) by Tuli *et.al.* (1988) for 50% Jy cross bred bulls. Slightly lower values (40.2%) were recorded by Mathew *et.al.* (1982) in case of 50% Brown Swiss bulls. 75% HF crossbred bulls showed significantly lowest P.T.M. value (23.54 ± 5.32%) whereas Mathew *et.al.* (1982) recorded higher over all mean value (49.39%) in 75% Brown Swiss bulls.

In 62.5% cross bred bulls PTM value (28.33 ± 2.35%) is similar to 75% Jy cross bred bulls in this study, which is much lower than in 62.5% Brown Swiss bulls (44.79%) studied by Mathew *et.al.* (1982).

Discussion

Post-Thaw motility is the main criteria for freezability of semen. Jy pure bred bulls were significantly superior to all other genetic groups. Capacity of spermatozoa to with stand the rigors of freezing at ultra low temperatures (-196°C) differs from bull to bull and more so in case of crossbred bulls.

Freezability and semen quality of Jy Crossbred bulls was found to be superior to HF crossbred bulls and this fact was further substantiated by rejection of tested HF and Jy cross bred bulls as their semen was not freezable. There was no rejection in case of 14 Jy pure bred bulls. But in the other category of crossbred bulls, the rejection percentage was 83.34% in 75% Jy, 44.5% in 62.5% Jy, 75.87% in 50% Jy, 87.5% in 75% HF and 78.58% in 62.5% HF cross bred bulls. 62.5% and 50% Jy cross bred bulls, exhibited higher percentage of freezability (44.16 and 42.65%) and lower rejection rate (44.5 and 66.99%)

Out of total 80 bulls tested for freezability the semen of only 32 bulls (40%) was found to be freezable. Vlachos *et.al.* (1968) also found a considerable difference in freezability of semen in between the bulls. Roberts (1971) also reported that about 30% of the bulls reared as sires in A.I. station donated semen which did not freeze satisfactorily. The process of cross breeding itself probably affects the quality of semen (Mathew, 1974).

It may be concluded that genetic combinations of exotic and indigenous blood has something to do with freezability. Further detailed cyto-genetic studies are essential to solve this enigma of freezability of semen in cross bred bulls.

Acknowledgements

Authors wish to thank Shri V.P. Rane, I.A.S., Director of Animal Husbandry, Maharashtra State, Pune, Dr. S.G. Manwatkar, Joint Director of Animal Husbandry, Nagpur Region, Nagpur, Dr. M.V. Nisal and Dr. R.G. Radke both past and present Dy.Director of Animal Husbandry (Frozen Semen) Nagpur for their encouragement.

Table 1 : Mean, Standard Error and Coefficient of Variation of various semen quality Characteristics of bulls of different genetic groups.

Sr. No.	Characters.		Jersey pure breds.	75 percent Jersey bulls	62.5 percent Jersey bulls	50 percent Jersey bulls	75 percent Friesian.	62.5 percent Friesian.
1	Volume (ml)	Mean	2.79^{b}	2.23^{a}	3.73^{b}	3.24^{c}	3.99^{c}	3.10^{c}
		Range	(1-6)	(1-4.5)	(1-7)	(1-6)	(1.5-8.5)	(1-7)
		SE	0.15	0.18	0.24	0.11	0.24	0.15
		CV %	41.22	55.16	41.79	50.93	48.98	51.93
		N	56	44	55	209	64	108
2	Mass Activity (+0-5)	Mean	3.04^{d}	2.07^{b}	2.30^{c}	1.93^{b}	1.77^{a}	2.27^{c}
		Range	(2.5-4)	(0-3)	(0-3.5)	(0-4)	(1-3.5)	(0.5-3.5)
		SE	0.03	0.12	0.15	0.06	0.10	0.08
		CV	6.05	31.88	48.26	45.08	44.63	35.56
		N	56	28	54	193	62	106
3	Initial Motility (Percent)	Mean	72.14^{e}	47.50^{c}	52.07^{d}	37.24^{b}	30.24^{a}	46.69^{c}
		Range	(70-80)	(0-70)	(0-80)	(0-80)	(5-80)	(5-80)
		SE	0.65	4.62	3.49	1.77	2.94	2.23
		CV	6.80	51.58	48.97	66.11	76.45	49.11
		N	56	28	53	192	62	106
4	Post Thaw Motility (Percent)	Mean	47.14^{d}	28.33^{b}	44.16^{cd}	42.65^{c}	23.64^{a}	28.33^{b}
		Range	(40-60)	(5-50)	(20-70)	(20.00)	(5-45)	(5-50)
		SE	0.92	5.26	0.99	1.54	5.32	2.35
		CV	14.7	64.95	33.15	25.32	77.50	51.75
		N	56	12	24	49	11	39

Means bearing same superscript do not differ significantly from each other.

Table 2 : Analysis of Variance for testing differences among means of different genetic groups for Volume, Mass Activity, Initial Motility and Post Thaw Motility

Sr. No.	Character	Source of Variation	df.	Mean Squares
1	Volume	Genetic groups	5	21.43^{xx}
		Error	530	2.63
2	Mass Activity	Genetic groups	5	8.09^{xx}
		Error	493	0.68
3	Initial Motility	Genetic Groups	5	13864.26^{xx}
		Error	491	519.89
4	Post Thaw Motility	Genetic Groups	5	2679.15^{xx}
		Error	185	153.89

xx = P<0.01.

REFERENCES

Bakshi, S.A. (1980), Andrological Investigations of cross bred bulls. M.V. Sc. Thesis in Animal Reproduction, P.K.V. Akola.

Baburao, K and Rao, A Rammohan, (1990). Body Scrotal, testicular Biometrics and Semen Characteristics among different genetic groups in 75% crossbred bulls Indian Vet. J. **67**:330-334.

Chauhan, F.S., Methrao, J.S. Takkav, D.D. and Singh, M. (1983) semen characteristics, deep freezing of semen and reproductive performance of crossbred cattle. Indian J.Dairy Sc. **36**:96-100.

Kohali, I.S. (1980). A report on the effect of certain climatological factors on semen quality of Jersey bulls stationed at Bikaner. 2nd All India Symposium on Animal Reproduction Aug. 27-29.

Mathew, Abraham (1974). Principles and Practice of Deep freezing Bull Semen. Pub. Indo-Swiss Project, Mattupatti, 685616, Munnar, Kerala PP 57.

Mathew, Abraham (1981) Seminar on "Importance of Artificial Insemination Technology for crossbreeding cattle in India" compendium: PP 63.

Mathew, Abraham, Joseph, P.J. and Jose, T.R. (1982) Semen characteristics of purebred and crossbred bulls. Indian Vet. J. **59**:364-367.

Nair, K. and Varadarajan, M. (1985). Daily sperm production of 50% Brown Swiss crossbred bulls, Kerala J. Vet. Sci. **16**:21-26.

Roberts, S.J. (1971). Veterinary obstetrics and Genital Diseases (Theriogenology), 2nd Edn. Pub J. Sinha. Scientific Book Agency, Calcutta.

Sharma, I.J., Agarwal, S.P. Shukla, S.P. and Dwarkanath, P.K. (1986) Comparative study of Hormonal and seminal characters of crossbred bulls and Buffalo Bulls. Indian Vet. J. **63**:636-638.

Snedecor, G.W. and Cochran, W.G. (1967). Statistical Methods. 6th Ed. The Iowa State University Press, Ames, Iowa, USA.

Raja C.K.S.V. and Rao, A.R. (1983) Semen Characteristics of Brown Swiss Crossbred bulls in relation to age. Indian Vet. J. **60**:451-54

Roy, D.J. Bandopadhyay, S.K. Biswas, J.C. and Bhat, P.N. (1975). Study on freezability of spermatozoa in three genetic groups of crossbred bulls Indian J.Anim.Hlth. **14**:67-41.

Tomar N.S., Sharma, K.C. and Shukla, S.N. (1985) Semen production in relation to the age of HF x Hariana bulls. Indian Vet. J. **62**:449-501.

Tuli R.K. Batabyal, A.K. Singal, S.P. and Razdan, M.N. (1988). Seminal characteristics and deep freezing of crossbred bulls semen using Tris-yolk glycerol Extendor Ind. J.Ani.Rep. **9**:16-18.

Vlachos, K. Tsakal, J.P. and Koutasouris (1985) Results of A.I. carried out in Greece Anim.Breed. Abst. 37:2543.

IJAR:11:2:85-88:1990

Studies On The Effect of Additives To Semen Diluent

M.R. BHOSREKAR[1], J.R. PUROHIT[2], and B.R. MANGURKAR[3],
Bharatiya Agro-Industries Foundation, Uruli-Kanchan-412 202 (Maharashtra)

ABSTRACT

Semen was diluted in Tris-Fructose-Citric acid eggyolk Glycerol diluent containing 7 mm. caffeine puris and 0.5% of detergent mixture of sodium and Triethenolamine lauryl sulphate. Control diluent was kept, and semen was split in three equal parts to be added to control and two treatment diluents. Glycerol was incorporated in the diluent at the rate of 6.4% for cattle semen to be added to semen in one step, while Glycerol was split and added to two portions at the rate of 3% and 11% to make 7% in final dilution for buffalo semen. The Glycerolization was done in 3 steps after cooling diluted buffalo semen to 5°C. The equilibration for cattle and buffalo semen was 6 hours and 3 hours respectively. The post-thaw forward motility was significantly higher in diluent containing detergent mixture as compared to caffeine puris or control.

• • •

In the past, several additives to semen diluents have been tried to improve the motility of spermatozoa as well as fertility of semen. Glycine, fructose and chelating agents (like EDTA, DEDTC, Cysteine hydrochloride and glutathione) to yolk and Milk-based diluents have been tried with variable results (Sengupta, *et.al.* 1969; Tomar, *et.al.* 1979). Amino acids and enzymes have been used with advantage (Hafs, 1968; Kirton and Hafs, 1965; Kirton, *et.al.* 1968; Hafs, 1971; Foulkes and Goody, 1980; Bhavsar, *et.al.* 1989).

Recently, Miyamoto and Nishikawa (1979), Singh *et.al.* (1986), Hukeri (1989) and Gehlout and Srivastava (1988) have used caffine, while Arriola (1982), Ahmed and Foote (1986), Arriola and Foote (1987) and Arriola (1987) used surfactants as additives to semen diluents in order to improve the post-thaw motility (PTM) and fertility of frozen semen.

Materials and Methods

Caffeine puris and surfactants, a mixture of Sodium and Triethanolamine Lauryl Sulphate (STLS) were used in Tris Citirc acid fructose-eggyolk-glycerol diluent in order to study their effect on PTM and fertility of Holstein Fresian, Jersey and buffalo semen.

Three adult bulls each of Holstein fresian, Jersey and Murrah buffalo were selected. All these bulls had good semen quality as well as freezability of Semen. The Semen from each bull was split in three equal parts one diluted to control diluent while other two parts were diluted to diluents containing 7 mm caffeine puris and 0.5% mixture of sodium and triethanolamine Lauryl sulphate. The semen extender used was Tris-fructose citric acid eggyolk-glycerol.

---

1. Research Programme Co-ordinator.
2. Joint Programme Co-ordinator.
3. Research Programme Organiser.

Glycerolisation procedure : Glycerol was incorporated initially in the extender at a concentration of 6.4% by volume. Extender was added to the semen at 35°C equal to the quantity of raw semen and allowed to cool to 22°C. After half an hour when the temperature of 22°C is attained, rest of the quantity of precooled dilutor is added to make final volume as per dilution rate to keep 30 x 10^6 sperm/dose. The semen was then packed in 0.3 ml minitubes by automatic filling and sealing machine (MT 65, Minitub to keep 0.28 ml of diluted semen). The filled straws are arranged horizontally on freezing racks and cooled to 5°C. After 6 hours of equilibration period the straws were frozen over Liquid Nitrogen by placing the racks on grill in static vapour column in LNR 320, (MVE) container. This procedure was adopted for exotic Jersey and Holstein Fresian semen. In case of buffalo semen, stepwise procedure of glycerolization was used in which the dilutor was split in two portions: one with 3% glycerol and 2nd with 11% glycerol. The extender having 3% Glycerol was added at 35°C equal to the quantity of raw semen. The diluted semen is then allowed to cool down to 22°C. After half an hour the pre-cooled dilutor containing 3% Glycerol was added to make half the final volume of the diluted semen and is transferred to cold handling cabinet at 5°C alongwith the portion of the dilutor containing 11% glycerol. After cooling for 1.5 hours and after attaining the temperature of 5°C by both the fractions, the dilutor containing 11% Glycerol was added to diluted semen in three equal portions at the interval of 10 minutes, slowly shaking in circular fashion. This procedure took 2 hours. The semen is then filled and sealed at 5°C in pre-cooled German Straws. The straws are arranged horizontally and cooled at 5°C for 3 hours equilibration time (which was found most suitable for buffalo semen, Bhosrekar, 1989) and frozen as per cattle semen.

Thawing and evaluation of frozen semen : Straws were individually thawed in water bath at 35°C for 30 seconds. The thawed semen was taken in 2 ml glass tubes kept at 35°C. The tube was then rotated in the palm to ensure thorough mixing. One drop of semen with the same straw is taken on the warm slide, covered with cover slip and examined under the phase contrast microscope with 20 phase having warm stage (Biotherm). At least 20 different fields were examined to assess the progressive motility. The percentage of initial, prefreezing and post freezing motility were transferred in to Archsin transformations and they were analysed for Analysis of variance.

Results and Discussion

It is evident that treatment affected the post freezing motility significantly ($P<0.01$), in all the breeds studied (Table 1). Additions of detergent mixture to the diluent has resulted in higher post thaw forward motility to the tune of 4 to 6% while caffeine puris in diluent could not enhance the motility after thawing (Table 2).

The increased post thaw motility in frozen semen diluted in diluent containing detergent mixture could have happened because of the action of surfactants on sperm cell membrane, making them more permeable and less sensitive to osmotic and glycerol shock during freezing and thawing (Arriola and Foote, 1987). Caffeine puris did not give the same effect as claimed by Miyamoto and Nishikawa (1974) and Singh *et.al.* (1986) and this could be because they studied its effect for preservation of liquid semen at 5°C and not for freezing. However, Hukeri (1988) and Gehlout and Srivastava (1988) reported significantly higher post thaw motility in frozen buffalo semen with caffeine in diluent.

Table 1 : Mean values with standard error for semen parameters.

Breed	(n)	No. of Treatment Observations	Initial Motility Mean ± S.E.	Pre-freezing Motility Mean ± S.E.	Post-freezing Motility Mean ± S.E.	No. of Doses Frozen Mean ± S.E.
HF	(18)	Control	81.70 ± 0.43	74.80 ± 0.18	62.40 ± 0.43	78.50 ± 6.74
	(18)	Caffeine puris	81.70 ± 0.43	74.50 ± 0.25	58.70 ± 0.66	78.50 ± 6.74
	(18)	Detergent	81.70 ± 0.43	75.10 ± 0.26	64.50 ± 0.54	78.50 ± 6.74
Jer	(19)	Control	84.60 ± 0.67	73.10 ± 0.95	59.80 ± 0.69	79.26 ± 5.67
	(19)	Caffeine puris	84.60 ± 0.67	73.90 ± 0.33	56.90 ± 0.88	79.26 ± 5.67
	(19)	Detergent	84.60 ± 0.67	74.80 ± 0.17	66.40 ± 0.57	79.26 ± 5.67
Buff.	(21)	Control	81.00 ± 0.33	75.00 ± 0.00	60.20 ± 0.61	52.48 ± 4.50
	(20)	Caffeine puris	81.10 ± 0.34	74.60 ± 0.22	59.90 ± 0.78	52.30 ± 4.73
	(21)	Detergent	81.00 ± 0.33	75.00 ± 0.00	65.40 ± 0.64	52.48 ± 4.50

Table 2 : Analysis of Variance Mean sum of squares

Sr.No.	Source of Variance	DF	Initial Motility	Pre-Freezing Motility	Post Freezing Motility	No. of Doses Frozen
1	Bet. Breeds	2	116.212**	7.549	3.35	13944.27**
2	Bet. Treatments	2	00.101	3.80	251.44**	0.07
3	Bet. Breed X Treatment	4	00.079	1.78	14.24	0.07
4	Residual	164	4.25	2.59	8.36	611.32

** P< 0.01

Acknowledgements

Authors take this opportunity to thank the President Dr. Manibhai Desai for his keen interest in this study. They also thank Mr. N.L. Phadke for his help in analysis of the data.

REFERENCES

Ahmed, K. and Foote, R.H. (1986) Post-thaw survival and fertility of frozen bull spermatozoa treated with antibiotics and detergent J. Dairy. Sci **69**:535.

Arriola, J.(1982) Interaction of Formaldehyde and of sodium and triethenolamine lauryl sulphate on the motility and fertilizing ability of rabbit and bull spermatozoa frozen in eggyolk and milk extenders Ph.D. thesis, Cornell University, Ithaca N.Y.

Arriola J. and Foote, R.H. (1987) Glycerolization and thawing effects on bull spermatozoa frozen in detergent treated eggyolk and whole egg extenders J. Dairy Sci. **70** : 1669-1670

Bhavsar, B.K.; Dhami, A.J. and Kodagali, S.B. (1989) Role of semen additives in improving fertility rates of Mehsana Buffalo semen. Indian Vet. J.**66** (7): 623-625.

Bhosrekar M.R. (1989) Upgrading of frozen semen technology and its transfer for development of buffaloes. Half-yearly report IDRC-Bis project for the period April to Sept. 1989.

Foulkes, D.A. and Goody, R.G. (1980) Bovine fertility following artificial insemination with semen frozen in diluent containing B-amylase and B-glucoronidase. Brit. Vet.J. **136**:331-338.

Foote, R.H. and Arriola, J. (1987) Motility and fertility of bull sperm frozen-thawed differently in eggyolk and milk extenders containing detergents. J.Dairy Sci. **70**:2642-2647.

Gehlout, B.S. and Srivastava, R.K.(1987) Effect of caffine citrate on post thawed motility of buffalo bull spermatozoa. Theriogenology **28**(6):767-772.

Hafs, H.D. (1968) Capacitation of spermatozoa and fertility of bull sperm with added amylase proc. 2nd Tech. Ent. Artif. Insem and Reprod. p.22 NAAB.

Hafs, H.D. (1971) A research and Development challenge Proc. Sliv. Anniv. Conv. NAAR.

Hukeri V.B. (1989) Deep freezing of Buffalo bull semen with additions of caffeine in tris dilutor. Proc. Indo French Joint Technical Seminar Feb. 11 to 13. BAIF, Uruli-kanchan-412 202, Pune.

Kirton, K.T. and Hafs, H.D. (1965) Sperm capacitation by uterine fluid or B amylase in Vitro. Sci. **150**:618-620.

Kirton, K.T., Boyd, L.J. and Hafs, H.D. (1968) Fertility of bull semen with added amylase J.Dairy Sci. **51**:1426-28.

Miyamoto, H. and Nishikawa, Y. (1979) Effect of caffeine on motility of bull spermatozoa. Japanese J. Zootech. Sci. **50**(9):601-608.

Sengupta, B.P.; Singh, L.N. and Roy, A. (1969) Preservation of buffalo spermatozoa in diluents supplemented with certain additives. Indian J. Anim. Sci. **39**:281-85.

Siingh, S.D.; Ansari, M.R. and Benjamin, B.R. (1986) Effect of caffeine on motility of buffalo spermatozoa in Vitro. Andrologia **18**(1):33-36.

Tomar, N.S., Sharma K.C., Sharma, K.B., Srivastav, K.N. and Singh B.P. (1979) The role of ascorbic acid and Cysteine hydrochloride on fertility rates of Liquid semen. Indian Vet J. **56**(3):163-167.

IJAR:11:2:88-91:1990.

Effect of Various Cooling Rates on Freezing Of Bull Semen*

A.C. MATHUR[1], B.P. BENJAMIN[2] and K.L. SAHNI[3]

Animal Reproduction Division, Indian Veterinary Research Institute, Izatnagar-243122 (U.P.)

ABSTRACT

Direct placement of straws filled at room temperature with yolk-tris-glycerol diluted Bull semen in water baths maintained at various temperatures ranging from 20°C to 5°C for storage periods of 15 or 30 minutes were assessed for post-thaw results. In all, seven treatments were tried. The differences between treatments and between bulls were highly significant. However, on application of resistance tests such as incubation and post-aging at 5°C, the differences between treatments were not significant. It is concluded that semen straws may be placed directly in water bath at 10°C and then gradually reduce the temperature to 5°C in 30 minutes or even 60 minutes for obtaining optimum post-thaw results. This would reduce the operational time considerably resulting in more efficiency in commercial frozen semen technology.

Cooling of diluted semen from around 35°C to 5°C constitutes a major time consuming component of semen freezing technology. A significant saving would result if this time could be shortened without reducing the fertility of the sample (Weidler and Zaugg, 1975). The present experiment was carried out as part of a study to develop some rapid test freezing method capable of giving results within 45 minutes (Mathur, 1989). An earlier experiment conducted in this regard had indicated that the straws immediately after filling with diluted semen (30°C) could be directly placed at temp. of 20°C, 10°C or even at 5°C without having any significant effect on prefreeze motility. In pursuance of the above observation the present study was conducted to find out the minimum time (of less than 30 min.) required either in storing the diluted sample at 20°, 10° or 5°C or in

* Part of M.V.Sc. Thesis. 1. P.G. Student. 2. Principal Scientist. 3. Head of Division.

cooling the sample to 5°C from 20°C and 10°C for the best post-thaw results.

Materials and Methods

The study involved 15 ejaculates from 5 Holstein-Friesian bulls (3 per bull). Every time only the first ejaculate having more than 40% initial motility were studied. The semen immediately after collection and intiaial evaluation was diluted with Tris-egg yolk-glycerol dilutor (containing 20% egg-yolk and 7% glycerol) at 30°C to have 60 million sperm/ml. The diluted semen was filled in 0.5 ml. French straws (Total 28 straws) and sealed with polyvinyl powder. Immediately after sealing, the straws were given the following seven different treatments for cooling to 5°C taking 4 straws per treatment :-

1. **Treatment 1 (T1) :** Involved direct placement of straws at 20°C followed by its cooling from 20°C to 5°C in 30 minutes.
2. **Treatment 2 (T2) :** Involved direct placement of straws at 20°C follwed by its cooling from 20°C to 5°C in 30 minutes.
3. **Treatment 3 (T3) :** Involved direct placement of straws at 20°C and its storage at 20°C for 30 minutes.
4. **Treatment 4 (T4) :** Involved direct placement of straws at 10°C followed by their cooling from 10°C to 5°C in 15 minutes.
5. **Treatment 5 (T5) :** Involved direct placement of straws at 10°C followed by their cooling from 10°C to 5°C in 30 minutes.
6. **Treatment 6 (T6) :** Involved direct placement of straws at 10°C and its storage at 10°C for 30 minutes.
7. **Treatment 7 (T7) :** Involved direct placement and storage of straws at 5°C for 30 minutes.

Procedure : *For Treatments 1, 2, 4 and 5.* Immediately after filling and sealing, the straws were transferred to different plastic rectangular 'Bread Boxes' (22cm x 11 cm) containing varying quantities of water prepared at pre-determined fixed temperature :

The water quantities were standardized earlier to have the desired fall in temperature in stipulated time. The plastic boxes were then placed in the freezing chamber of the refrigerator for further cooling to 5°C (Sahni and Mohan, 1988 a,b).

For treatment number 3,6 and 7: Straws immediately after filling and sealing were transferred to three different plastic 'Bread Boxes' each containing about 400ml water of desired initial temp. (20°C, 10°C and 5°C for T3, T6 and T7 respectively). The temperature of 20°C and 10°C were maintained by the addition of Ice-cool or warm water (as per the need) while the box for T7 (5°C temp.) was placed in refrigerator at 5°C.

After the stipulated time, the straws were taken out from the respective boxes, one straw per treatment was used for assessing pre-freeze motility. The remaining three straws per treatment were transferred to cold handling cabinet (5°C) and were frozen in

Treatment No.	Quantity of Water taken	Initial Temp.of Water
T1	100ml	20°C
T2	200ml	20°C
T4	225ml	10°C
T5	325ml	10°C

liquid nitrogen by the two-step freezing method (Jondet *et.al.* 1980)

Thawing was done at 37°C for 30 seconds. After thawing, out of the 3 straws (per treatment) one straw was used for assessing post-thaw motility, second straw was kept in incubator (37°C) for one hour then assessed for post-incubation motility and the third straw was kept in refrigerator at 5°C (in thawing water) for 24 hrs and then assessed for post-ageing motility.

Results

The overall initial motility was 61.66 ± 2.27%, sperm concentration 842.00 ± 95.45 million/ml., percent dead sperm 25.73 ± 2.87 and percent abnormal sperm were 8.93 ± 0.43.

The results of motility assessment at prefreeze, post-thaw, post-incubation and post-ageing stages are presented (Table 1). The analysis of variance of the data was done by two way analysis. The significantly different treatment means were compared by critical difference among treatments.

For prefreeze motility, the differences between treatment were not significant but there was highly significant difference ($P<0.01$) between bulls.

For post thaw motility, there was highly significant difference ($P<0.01$) both between treatments and between bulls. Among treatments, the grading for post-thaw motility values were in the following sequence: T5(32.66 ± 2.75), T7(28.66 ± 1.85), T2(28.33 ± 2.95), T4(27.00 ± 3.37), T1(23.33 ± 2.61), T3(21.66 ± 2.47), T6(20.66 ± 2.33), but there was no significant difference between treatments giving post-thaw motility above 25% (T5, T7, T2 and T4).

For both post-incubation and post ageing motility, there were no significant differences between treatments but the differences were highly significant ($P<0.01$) between bulls for both these stress tests. The motility values for both the tests were highest for T5 (like post-thaw motility) and minimum for T3.

Table 1 : Mean motility values of cattle semen after application of different cooling treatments at different evaluation stages.

Evaluation Parameter	Mean Values (%) after different treatments.						
	T1	T2	T3	T4	T5	T6	T7
1. Prefreeze Motility.	53.66 ±2.41	54.00 ±2.81	56.33 ±2.51	53.33 ±3.22	55.66 ±2.33	53.33 ±2.37	45.66 ±2.52
2. Post-thaw motility	bc 23.33 ±2.61	ab 28.33 ±2.95	a 21.66 ±2.47	abc 27.00 ±3.37	a 32.66 ±2.75	c 20.66 ±2.33	ab 28.66 ±1.85
3. Post-incubation motility	11.26 ±2.41	12.73 ±3.28	6.66 ±1.97	12.26 ±3.27	16.53 ±3.95	10.33 ±2.49	10.73 ±2.40
4. Post-ageing motility	5.73 ±1.48	7.40 ±2.02	5.33 ±1.12	8.93 ±2.02	12.06 ±2.76	8.00 ±2.35	11.06 ±2.37

The values with no superscript indicate non-significant difference between treatments.

The overall mean values with identical superscript indicate non-significant difference between/among them.

Discussion

The non-significant difference between treatments for the prefreeze motility showed that direct placement of the straws to 20°C, 10°C and 5°C temperature and their subsequent maintenance at these temperatures for 30 minutes (T3, T6 and T7) as well as their further cooling to 5°C in 15 minutes (T1, T4) and 30 minutes (T2, T5) did not result in any appreciable damage to viability of sperm cells pre-freeze stage. In an earlier experiment (Mathur, 1989) it was observed that direct placement of straws to temperatures of 30°C, 20°C, 15°C, 10°C and 5°C for 15 minutes did not result in any significant difference in pre-freeze motility.

Post-thaw motility was highest for treatment T5 and lowest for T3, and T6. Further T5 was having non-significant difference with T7, T2 and T4. The values of post-incubation and post-ageing motility were also highest for T5. These observations indicated that :

i. Immediately after filling and sealing at room temperature the straws could be safely transferred to 10°C and even to 5°C without significantly affecting their pre-freeze motility.

ii. It was desirable to cool semen to 5°C (starting from direct placement temperatures of 20°C or 10°C) before its freezing for getting better post-thaw results.

iii. Direct placement of straws to 10°C and its subsequent cooling to 5°C (better in 30 minutes vs 15 minutes) gives better post-thaw result in comparison to direct placement at 20°C and its subsequent cooling to 5°C.

It is concluded that for obtaining optimum post-thaw motility for commercial semen freezing work, instead of starting cooling to 5°C from around 35°C, direct placement of straws immediately after filling (around 30°C) to 10°C and then its subsequent cooling to 5°C need to be tried which may reduce the time involved in cooling to 5°C. The results of post-incubation and post-ageing motility assessment had also confirmed the above findings.

REFERENCES

Jondet, R.; Rabadeux, Y and Jondet M (1980) Observation on freezability of bull ejaculates using a two-step freezing procedure. Proceedings 9th Internat. Cong. Anim. Reprod. and A.I., Madrid, Spain P-389.

Mathur, A.C. (1989) Studies on development of some rapid methods for test-freezing of bovine semen. M.V. Sc. thesis. I.V.R.I., Izatnagar.

Sahni, K.L. and Mohan, G. (1988a) A modified method of processing bovine semen for freezing under tropical conditions. Indian. J. Anim. Sci. 58(9); 1075.

Sahni, K.L. and Mohan, G. (1988b) A simplified method of freezing bovine semen for screening of bulls under field conditions. Indian J. Anim. Sci. 58(9): 1046.

Weidler, J.F. and Zaugg, N.L. (1975) Fertility of semen in straws unaffected by shortened cooling time to 5°C. A.I. Digest. 23(8): 9.

IJAR:11:2:92-95:1990

Studies On Some Characteristics Of Exotic And Crossbred Bull Spermatozoa *

D.M. SINGH and G.R. PANGAWKAR
Department of Obstetrics and Gynaecology, College of Veterinary Science,
Punjab Agricultural University, Ludhiana - 141 004.

ABSTRACT

Seminal studies were made by collecting at least ten ejaculates, each from 7 HF, 4 Jersey, 5 HFS (1/2 HF + 1/2 Sahiwal), 4 KF (1/2 HF + 1/4 BS + 1/4 Th) and 3 HRS (1/2 HF + 1/4 RD + 1/4 Sahiwal) bulls. The mean values for ejaculate volume, mass activity, initial motility, live sperm count and sperm concentration in HF, KF and HRS bulls were recorded.

The total abnormalities of sperm head, mid piece and tail including protoplasmic droplets were 10.01 ± 0.14% in HF, 10.45 ± 0.20% in Jersey, 8.48 ± 0.15% in HRS, 8.82 ± 0.16% in KF and 15.80 ± 0.23% in HRS bulls.

The mean values for different semen characteristics recorded in all breed groups were within the range stipulated for normal fertile bulls. A highly significant breed difference for volume, initial motility, live sperm count and total sperm abnormalities were recorded.

• • •

Exotic bulls are imported in this country in large number to upgrade indigenous cattle, ultimately to improve milk production. Raju and Rao (1982), Bhatt and Chauhan (1982) and Chinnaiya and Balkrishnan (1985) studied the semen quality of crossbred bulls. The performance of pure breds and crossbreds with particular reference to quality of semen under varying environmental conditions of the tropics has not been adequately studied. The semen characteristics of pure and crossbred bulls with different genetic make up were compared.

Materials And Methods

Semen was collected from 23 young mature bulls : 7 Holstein-Friesian (HF), 4 Jersey, 5 HFS (1/2 Holstein-Friesian + 1/2 Sahiwal), 4 Karan-Swiss Friesian (1/2 Holstein-Friesian + 1/4 Brown-Swiss + 1/4 Tharparkar) and 3 Holstein-Friesian-Red Dane Sahiwal (1/2 Holstein-Friesian + 1/4 Red Dane + 1/4 Sahiwal) stationed at Livestock Farm, P.A.U., Ludhiana and Semen Bank, Patiala. Ten to twelve ejaculates at regular interval from each bull were collected in artificial vagina.

Various semen characteristics : volume, mass activity, live sperm count and sperm concentration were recorded. Morphological abnormalities of spermatozoan head were studied in semen smears of each bull stained with William's stain, whereas percent acrosomal defects, free loose heads, mid piece defects, protoplasmic droplets and tail abnormalities were counted in buffered formal

* Part of the thesis submitted by the first author in partial fulfilment of M.V.Sc. degree to P.A.U. Ludhiana.

saline preserved semen samples under phase contrast microscope.

Statistical methods were used for all calculations as described by Snedecor and Cochran (1967).

Results And Discussion

The mean values of physical characteristics of semen in different genetic groups of bulls are presented in Table 1.

The mean ejaculate volume recorded in HF and Jersey bulls concurred with the findings of Holy (1971) and Chalapathi and Rao (1982). The difference in ejaculate volume with other workers could be due to the different agro-climatic conditions under which the different groups of animals were kept.

Among the crossbreds, HFS bulls recorded higher semen volume than purebreds which is in confirmity with the findings of Patel and Kodagali (1985) and Chinnaiya and Balkrishnan (1985). Triple crossbred KF bulls produced maximum semen volume which was higher than that reported by Verma *et.al.* (1985). In HRS bulls the volume characteristic was found to be in agreement with the findings of Chauhan *et.al.* (1983).

Analysis of variance revealed a significant breed difference in the ejaculate volume. In KF bulls, the ejaculate volume was significantly higher ($P<0.01$) than in the other breed groups.

The mean mass activity of semen in HF bulls was higher than that reported by Mohanty (1981). In Jersey bulls, mass activity corresponded to the values reported by Saxena and Tripathi (1979). The values in crossbred HFS and KF bulls were similar as reported by Chinnaiya and Reddy (1984). The mass activity exhibited in HRS bulls was quite lower as compared to that reported by Chauhan *et.al.* (1983). This might be due to the numerical scale 0 to 4 used in the present study. The mass activity was significantly higher in pure breeds and lower in triple crosses (KF and HRS), clearly indicating breed differences.

The mean initial sperm motility in HF and Jersey bulls corresponded to the values reported by Mohanty (1981) and Bujarbaruah *et.al.* (1982). Higher initial motility as compared to other reports in pure, crossbred and triple crossbred bulls could be due to higher environmental temperatures encountered (Rao and Rao, 1975). This could also be due to the variations in the seminal plasma composition, since the latter is known to affect the spermatozoan motility (Ganguli, 1978).

The live sperm count recorded in HF and Jersey semen was in agreement with the findings of Pangawkar (1983) and Raju and Rao (1982) respectively. In HFS and KF bulls the values were in agreement with the findings of Biswas *et.al.* (1976) and Roy *et.al.* (1975) respectively. The live sperm percentages in HRS breed was found to be lowest among the breed groups studied. Significant differences ($P<0.01$) were obtained between different genetic groups of bulls. The live sperm percentage was higher in HF and HFS bulls ($P<0.05$) and low ($P<0.01$) in HRS bulls.

The mean sperm concentration in HF and Jersey breed was close to the values reported by Pangawkar (1983) and Chalapathy and Rao (1982). Almost similar values were recorded in HFS and KF bulls. The present results in HRS were found to be lower than that of Chauhan *et.al.* (1983). This might be due to the age difference of bulls. Significant

variations in the sperm concentration between the bulls as well as between the breeds were recorded, which could be due to the different genetic combinations in breed groups of bulls.

The incidence of head defects, free loose heads, mid piece defects and tail defects in HF and Jersey bulls are within normal limits as per the observations of Raju and Rao (1982). In HFS breed, the sperm defects were quite lower and were in consonance with Raja and Rao (1983). In triple crossbred KF and HRS bulls, the latter exhibited higher sperm abnormalities, when compared with other genetic groups under study. The findings of higher sperm abnormalities accompanied with lower incidence of initial motility and live sperm count in HRS bulls was supported by the observations of Pangawkar (1983) who recorded a significant negative correlation of sperm abnormalities with initial motility and live sperm counts. Among the sperm abnormalities studied in all breed groups, the head defects were higher than mid piece and tail defects. However, the total sperm abnormalities in all breeds were found to be within permissible limits as stipulated by Lagerlof (1934).

Acknowledgements

The authors are thankful to Dr. R.D. Sharma, Prof-cum-Head, Department of Obstetrics and Gynaecology and Dr. M.S. Tiwana, Head, Department of Animal Science for providing necessary facilities to carry out research work.

REFERENCES

Bhatt, V.K. and Chauhan, R.A.S. (1982) Note on certain physical and microscopical characteristics of semen of Red Dane and Red Dane x Rathi bulls. Indian J. Anim. Sci. **52** : 259-60.

Biswas, J.C., Raina, B.L., Kumar, R. and Bhat, P.N. (1976) Studies on live and abnormal spermatozoa in crossbred bulls. Indian J. Anim. Health. **15** : 129-132.

Bujarbaruah, K.M., Rajkonwar, C.K., Borgahain, B.N. and Kakati, B.N. (1982) Note on semen characteristics in young Jersey and Red Dane bulls. Indian J. Anim. Sci. **52** : 589-593.

Chalapathy, P.V. and Rao, A.R. (1982) Effect of imrpoved housing on semen characteristics of Jersey bulls. Indian Vet. J. **59** (4) : 274-280.

Chauhan, F.S., Matharoo, J.S., Takkar, O.P. and Singh, Mehar. (1983) Semen characteristics, deep-freezing of semen and reproductive performance of crossbred cattle. Indian J. Dairy Sci., **36** (1) : 96-100.

Chinnaiya, G.P. and Balakrishnan, M. (1985) Studies on characteristics of crossbred bull semen and its keeping quality. Indian J. Anim. Reprod. **6** (2) : 71-74.

Chinnaiya, G.P. and Reddy, P.S. (1984) Motility index : A simple and reliable test for evaluating semen quality. Indian J. Anim. Reprod. **5** (2) : 64-67.

Ganguli, N.C. (1978) Role of seminal plasma in regulation of sperm motility. Symposium on "Recent Advances in Reproduction and regulation of Fertility", New Delhi.

Holy, L. (1971) Production and equality of semen in Brown Swiss and HF bulls in sub-tropical conditions in Cuba. Vet. Med. Praha **16** : 501-512 (Anim. Breed. Abstr. **40** : 3121).

Lagerlof, N. (1934) Morpholoysche unter Suchungenuler Varanderugun in Spermabid und in den Hoden bei Bullen mit. Verminderter order Suffgehobner Pertilitat. Acta. Path. Microbiol. Scand. Suppl. 19.

Mohanty, D.N. (1981) Semen characteristics and their correlations with testicular and body measurements in Holstein-Friesian bulls. M.V.Sc. thesis, Punjab Agril. University, Ludhiana.

Pangawkar, G.R. (1983) Biochemical and cytomorphological studies on normal and subfertile bull semen. Ph.D. thesis, Punjab Agricultural University, Ludhiana.

Patel, K.V. and Kodagali, S.B. (1985) Diagnostic and investigative andrology in crossbred bulls. M.V.Sc. thesis abstract. Indian J. Anim. Reprod. **6** (2) : 107-110.

Raja, C.K.S.V. and Rao, A.R. (1983) The semen characteristics of Brown Swiss crossbred bulls in relation to age. Indian Vet. J. **66** : 451-454.

Raju, M.S. and Rao, A.R. (1982) Note on the semen characteristics of crossbred and purebred bulls. Indian J. Anim. Sci. **52** : 1230-32.

Rao, R.M. and Ramamohana Rao, A. (1975) Studies on semen characteristics of Tharparkar and Jersey bulls. Indian Vet. J. **52** (12) : 889-900.

Roy, D.J., Biswas, J.C., Bandhypathy, S.K. and Bhat, P.N. (1975) Study on the freezability of spermatozoa in genetic groups of crossbred bulls. Indian J. Anim. Health **XIV** (I) : 67-71.

Saxena, V.B. and Tripathi, S.S. (1979) A note on abnormal spermatozoa in semen of Jersey bulls. Indian J. Anim. Sci. **49** : 738-740.

Snedecor, G.W. and Cochran, W.G. (1967) Statistical Methods. 6th Ed. The Iowa State University Press, Ames, Iowa, USA.

Verma, R.P., Kalicharan, Greesh Mohan and Benjamin, B.R. (1985) A comparative study on the seminal attributes of normal and abnormal crossbred bulls. Indian Vet. Med. J. **9** : 236-238.

Table 1 : Mean values of physical characteristics of semen and sperm abnormalities in exotic and crossbred bulls (mean ± SE).

Genetic Group	Volume (ml)	Mass activity (0-4)	Initial motility (%)	Live sperm (%)	Sperm concentration (millions/ml)	Total sperm abnormalities (%)
HF (7)	6.85 ±0.15	3.10 ±0.54	79.41 ±0.38	86.75 ±0.41	1227.85 ±29.75	10.01 ±0.14
Jersey (4)	5.75 ±0.97	3.00 ±0.11	76.57 ±0.90	82.97 ±0.90	1157.75 ± 38.75	10.45 ±0.20
HFS (5)	7.00 ±0.22	2.88 ±0.08	79.74 ±0.64	86.40 ±0.48	1191.80 ± 53.77	8.48 ±0.15
KF (4)	8.63 ±0.45	2.83 ±0.06	76.77 ±1.04	82.12 ±0.92	1148.25 ± 30.23	8.82 ±0.16
HRS (3)	3.85 ±0.24	2.80 ±0.06	67.49 ±0.90	74.53 ±1.04	1181.00 ± 42.25	15.80 ±0.23

Figures in parenthesis represent the number of ejaculates.

Table 2 : Analysis of variance of the seminal characteristics in different genetic groups of bulls.

Source of variation	d.f.	Volume	Mass activity	Initial motility	Live sperm count	Sperm concen-tration	Total sperm abnormalities
Between breeds	4	31.12**	0.15	247.10**	242.91**	297.11	86.00
Within breeds	9	0.65	0.04	10.53	5.58	180.79	0.55
Error	36	0.66	0.06	5.54	6.47	153.47	0.36

** $P<0.01$

IJAR:11:2:96-99:1990

A Comparative Study of Acrosomal Morphology Of Crossbred And Holstein-Friesian Bull Semen.

M.L. SHARMA[1], G. MOHAN[2] and K.L. SAHNI[3]

Division of Animal Reproduction, Indian Veterinary Research Institute, Izatnagar-243 122 (U.P.)

ABSTRACT

12 ejaculates each of six triple crossbred bulls and 12 random samples of three HF bulls, were studied. The average values of intact acrosome in Fresh semen samples were 74.05 ± 1.19% and 89.41 ± 0.96% in crossbred and HF bulls respectively. Significant differences between breeds and between bulls were observed. Similar variations were seen in the post thawed semen. The average values were 69.40 ± 1.29% and 80.00 ± 1.13% in the two breed groups. The number of denuded acrosomes was significantly higher in post thawed semen. The extent of acrosomal damage was not significant between fresh and post thawed semen.

* * *

Nearly 30-40 percent of crossbred bulls produced semen of poor quality under tropical conditions (Sahni and Mohan, 1988). The objective of the present study was to estimate the extent of damage in Acrosomal content of sperms both in fresh and frozen (thawed) stages in crosses and make comparisons with pure Holstein bulls under identical conditions of feeding and management.

Saake *et.al.* (1968) examined spermatozoa of Jersey and Holstein bulls in Giemsa stained preparations and recorded the incidences of Knobbed, ruffled and incomplete acrosome as 5,8 and 20% respectively. Wells *et.al.* (1969) observed that the ejaculates from 22 bulls in sexual rest averaged 21% acrosomal abnormalities. Healey (1969) reported identical ultra structure of bull sperm before and after freezing. Reza (1985) reported that months of collection or type or storage caused no significant effect on the percentage of damaged acrosome, but there was highly significant difference between bulls. Aalseth and Saacke (1986) reported that the acrosomal integrity was not altered by staining and there was high correlation of live percent spermatozoa and percentage of intact acrosomes.

Materials And Methods

Six triple crossbred bulls (1/2 HF x 1/4 J x 1/4 H) and Holstein-Friesian bulls were taken for the present study. The study was conducted in Germ Plasm Centre of I.V.R.I., Izatnagar from October 1987 to February 1988. The collection was taken on alternate days, on an average 12 collections from each cross bred bull and 4 collections from each Holstein-Friesian bulls were taken (Total 12).

The acrosome integrity (PIA) based on acrosomal damage in Fresh and Frozen semen was studied in Giemsa stained smears

1. Veterinary Officer C/o Vety. Hospital, Kullu-175101 (H.P.)
2. Scientist (Sr.Gr.).
3. Head of the Division

(Watsen, 1975). The acrosomal abnormalities were classified as Knobbed, Swollen, denuded, incomplete and ruffled, based on the ultrastructure of the acrosome.

Results

The percentage of intact acrosome in freshly collected semen are presented in Table 1. The corresponding values of acrosomal damage are also presented in Table 2.

The mean values of acrosomal integrity in crossbred and Holstein-Friesian bulls were 74.05 ± 1.19% and 89.41 ± 0.96% respectively. The values ranged between 35 and 88% and 84.5 and 95% respectively. The percent acrosomal damage in crossbred bulls varied widely. The mean values (%) of Knobbed, Swollen, denuded, incomplete and ruffled acrosome were 2.39 ± 0.31, 2.65 ± 0.42, 13.10 ± 1.28, 3.51 ± 0.41 and 3.37 ± 0.41. The mean values (%) of acrosomal damage in Holstein-Friesian bulls (Pooled) viz. Knobbed, Swollen, denuded, incomplete and ruffled were 0.87 ± 0.28, 1.87 ± 0.28, 4.70 ± 0.42, 2.08 ± 0.49 and 1.08 ± 0.30 respectively.

Acrosomal scorings were made 24 hours after freezing of semen samples. The PIA in post-thaw samples are presented in Table 2. The mean values of acrosomal damage are also presented in Table 2

The mean values of intact acrosome (%) 24 hours post-thaw in crossbred and Holstein-Friesian bulls were 69.40 ± 1.29, and 80.00 ± 1.13 respectively. The mean values (%) of acrosomal damage viz; Knobbed, Swollen, denuded, incomplete and ruffled in these two breed groups were 2.97 ± 0.43, 3.63 ± 0.37, 15.21 ± 1.15, 4.21 ± 0.38, and 3.80 ± 0.50 and 0.95 ± 0.19, 1.83 ± 0.17, 6.75 ± 0.02, 3.29 ± 0.52 and 1.16 ± 0.33 percent respectively.

Discussion

The average value (%) for corssbred (74.05 ± 1.19) was lower than that of HF (Pooled) bull semen samples (89.41 ± 0.96) in fresh semen, which did not differ significantly from each other. The considerably lower values observed in two crossbred bulls (No. 317 x 2 and 622 E2) has created the significant difference between the average values of the two breed groups. However the values observed in our study were lower than that reported by Saacke *et.al.* (1968). The total acrosomal damage in crossbred bulls was comparable with the results of Wells and Awa (1970), who reported 12.7 to 29.3% acrosomal damage. The variation of percent intact acrosome was significant ($P \leq 0.01$) between the bulls (Table 3). The variation in acrosomal damage between the bulls could be due to the variability of response in the cell membrance and some primary defects in spermatogenesis.

The mean values (%) of intact acrosomes, 24 hour post thaw were higher in HF bulls (86.00 ± 1.13) than the crossbred bulls (69.9 ± 1.29). The extent of acrosomal damage was not significant between Fresh and post-thawed semen.

Acknowledgement

We thank Dr. P.N. Bhat, Director, I.V.R.I., for providing the facilities.

Table 1 : Mean ± S.E. of PIA and Acrosomal damage in freshly collected semen of crossbred and H.F. bulls.

Bull No.	No. of Observation	PIA (%)	ACROSOMAL DAMAGE (%)				
			Knobbed	Swollen	Denuded	Incomplete	Ruffled
1222 X_2	12	83.05 ± 0.41	1.72 ± 0.18	2.22 ± 0.20	7.55 ± 0.34	2.25 ± 0.18	3.16 ± 0.20
1186 X_2	12	83.00 ± 0.45	2.32 ± 0.62	1.67 ± 0.20	7.96 ± 0.46	2.67 ± 0.23	2.96 ± 0.34
317 X_2	12	45.90 ± 0.88	4.25 ± 0.21	3.50 ± 2.70	32.70 ± 2.70	6.91 ± 0.12	6.75 ± 0.73
622 E_2	12	61.45 ± 2.78	2.91 ± 0.31	3.79 ± 0.41	24.64 ± 3.73	4.56 ± 0.86	4.20 ± 0.59
693 E_2	12	83.85 ± 0.74	2.22 ± 0.30	3.31 ± 0.96	6.95 ± 0.75	4.16 ± 0.49	2.20 ± 0.17
934 E_2	12	81.25 ± 0.84	2.45 ± 0.31	2.25 ± 0.44	7.25 ± 0.58	3.95 ± 0.50	3.29 ± 0.57
Overall (Crossbred)	72	74.05 ± 1.15	2.39 ± 0.31	2.65 ± 0.49	13.10 ± 1.28	3.51 ± 0.41	3.37 ± 0.41
H.F.(Pooled)	12	89.41 ± 0.96	0.87 ± 0.28	1.87 ± 0.42	2.08 ± 0.49	2.08 ± 0.49	1.04 ± 0.30

Table 2 : Mean ± S.E. of PIA and Acrosomal damage (24 hour post freeze) of crossbred and H.F. bulls.

Bull No.	No. of Observation	PIA (%)	ACROSOMAL DAMAGE (%)				
			Knobbed	Swollen	Denuded	Incomplete	Ruffled
1222 X_2	12	81.05 ± 0.41	2.80 ± 0.99	2.58 ± 0.16	9.05 ± 0.39	2.77 ± 0.15	3.46 ± 0.18
1186 X_2	12	80.55 ± 0.41	2.25 ± 0.28	2.21 ± 0.15	9.25 ± 0.32	2.82 ± 0.22	3.32 ± 0.42
317 X_2	12	39.50 ± 0.76	3.70 ± 0.34	5.08 ± 0.47	28.58 ± 3.95	5.73 ± 0.59	4.66 ± 0.49
622 E_2	12	53.05 ± 3.08	4.50 ± 0.32	4.08 ± 0.50	38.12 ± 0.88	7.95 ± 0.76	6.58 ± 0.98
693 E_2	12	79.70 ± 0.43	2.25 ± 0.23	2.95 ± 0.29	9.04 ± 0.53	2.96 ± 0.44	3.06 ± 0.41
934 E_2	12	77.50 ± 0.80	2.37 ± 0.25	3.08 ± 0.39	8.66 ± 0.49	4.00 ± 0.67	4.37 ± 0.69
Overall (Crossbred)	72	69.40 ± 1.29	2.97 ± 0.43	3.63 ± 0.37	15.28 ± 1.15	4.21 ± 0.38	3.80 ± 0.50
H.F.(Pooled)	12	86.00 ± 1.13	0.95 ± 0.19	1.83 ± 0.17	6.75 ± 0.62	3.29 ± 0.52	1.16 ± 0.33

Table 3 : Analysis of variance (M.S.S.) showing the variation among percent intact acrosome and acrosonal damage.

Source of Variance	d.f.	PIA (fresh semen)	PIA (Post-thaw)
Between age groups	1	185.88	234.87
Between bulls	4	1428.29**	1707.35**
Error	66	20.45	22.61

Note : The percentage values have been transformed into arcsin percentage for ANOVA.
PIA, percent intact aerosome.
** Significant at 1% level.
* Significant at 5% level.

REFERENCES

Aalseth, E.P. and Saacke, R.G. (1986). Vital staining and acrosomal evaluation of bovine spermatozoa. Gamete Research. **15** (1) : 73-81.

Healey, P. (1969). Effect of freezing on ultra-structure of the spermatozoa of some domestic animals J. Reprod. Fert. **18** : 21-27.

Reza Guevara, G (1985). Acrosomal damage of bull semen frozen in french straws and in pipettes. ABA. **54** : No. 6487.

Sahni, K.L. and Mohan G. (1988). Annual Report. Animal Reproduction Division, I.V.R.I. Izatnagar.

Saccke, R.G., Amann, R.P. and Marshall, C.E. (1968). Acrosomal cap abnormalities of sperm from sub fertile bulls. J. Anim. Sci. **27**:1391-1400.

Watson, P.F. (1975). Use of Giemsa stain to detect changes in acrosome frozen ram spermatozoa. Vet. Rec. **97**:12-15.

Wells, M.E., Awa, O.A. and Wonda Frash T. (1969). Evaulation of acrosomal morphology with the Wells-Awa stain. J. Dairy. Sci. **52**:563.

Wells, M.E. and Awa, O.A. (1970) New technique for assessing acrosomal characteristics of spermatozoa. J. Dairy Sci. **53** : 227-232.

IJAR:11:2:99-104:1990

Studies On Artificial Insemination in Equines

Lt Col. N. M. SINGHVI.

Remount Training School and Depot, Saharanpur-247 001 (U.P.)

ABSTRACT

Artificial insemination in equines has been carried out for the first time in India. Ten Horse Stallions, Ten Donkey Stallions and 468 mares of three distinct groups were taken. Method of semen collection and insemination is described. The conception rate (C.R.) was 39.13% while that in mule breeding reached 56%. There was significant difference in C.R. of mares inseminated in one oestrus and those inseminated in more than one oestrus.

* * *

Of all the domesticated animals, horse received minimum attention for artificial

breeding. Horses are registered on the basis of pedigree performance. If not properly controlled, A.I. allows an opportunity for dishonesty and chicanery. Hence, AI in thorough breds has not been recommended. Certain breeding societies still have legislation preventing the use of AI in horse breeding. Thorough bred breeder association has, however, lately advocated the introduction of AI under strict supervision of veterinarians and the stud book authority, subject to confirmation of parentage by blood grouping. This has been done on the belief that it would provide effective means of national and international control of infectious diseases including CEM (Anon, 1978).

AI is extensively used in China, Russia and many East European countries. It is also in vogue in Japan, Germany, Poland, Holland, Scandinavia, America and Australia (Allen *et.al.* 1976) but remains a virgin field in India. Sane and his co-workers, did make an attempt in 1953 with the object of over coming sub fertility in horses. The work however was limited to sporadic cases and results were not encouraging. The results of AI in foreign countries have been reported to be as high as 67 to 70%. Allen *et.al.* (1976) obtained C.R. of 60 percent by one single insemination done within one hour of collection of semen stored at 30-35°C. The results increased upto 92% by three inseminations. Petelikova *et.al.* (1977) reported C.R. of 66.6% in mares inseminated with frozen semen within 12 hrs. of estimated ovulation. Merkt (1984) reported pregnancy rate to AI as 66% for non-milking and 50% for milking mares, while Muller (1987) reported average number of inseminations per oestrum to be 2.2, with 56% pregnancy rate and 48% foaling.

India is having very few proven Stallions for natural service located at particular point at far flug distances. Since mares are scattered all over the country, breeders/owners lack access to Stallions at appropriate time. Artificial breeding, therefore, can be of great value both qualitatively and quantitatively.

Materials And Methods

Ten horse and 10 Donkey Stallions aged 5 to 10 years were taken for semen collection. A total of 468 mares belonging to three distinct groups were selected for breeding by A.I. thus :

(i) Horse breeding Brood Mares (HBBM) - 46

(ii) Heavy mule breeding Brood Mares (MAMBBM) - 262

(iii) Light mule breeding Brood Mares (GSMBBM) - 160

All these animals belonged to the army establishment, they were kept under controlled conditions providing similar management. Semen was collected by Colorado model AV. None of the Stallions had previous training to ejaculate semen in A.V. Two to three collections were taken from each Stallion in a week.

The Colorado model of AV consists of : (a) A casing made of heavy plastic, 16 cm in diameter and 55 cm in length. Casing has a valve assembly and a leather cover with handle. (b) Inner liner of thick latex 75 cm long and 19 cm in width. This is fitted in the casing to form a water jacket. (c) Combination liner and cone made of thick and strong latex about 70 cm long and 16 cm wide on one side and tapered on other side to accomodate the collection bottle. (d) Filter assembly - This includes collection bottle, filter, filter ring and radiator clamp. (e) Protector

cone - This is a cover made of cloth stuffed with cotton. It is placed over the collection bottle to protect the semen from exposure to temp. and sunlight.

Collection of Semen : This involves proper scheduling and sexual preparation of males as well as use of proper technique. The best known method for collection of semen is by artificial vagina because it stimulates natural conditions and evokes ejaculatory nerves of glans penis through vaginal walls.

Assembling the AV : The latex liner was inserted into the plastic casing keeping equal lengths of the liner on both sides. The liner was turned back over both the ends so that about 7-8 cm of liner could be turned on each sides. Rubber band was then placed on both the ends to prevent leakage of water and air. The combination liner and cone was then inserted through the inner liner and turned back over the front end of the casing about 8 cm. The tapered portion of the combination liner and cone emerged out about 2-3 cms. The collection bottle fitted with the filter assembly was then fixed on the tapering end of combination liner and a clamp fitted over the liner and collection bottle. The protective cone was placed over the bottle.

Preparation of AV: After assembly, the AV was filled with about 3.5 to 4 ltr of warm water at 55-60°C. It now weighed about 8 kg. The amount of water was regulated to create required pressure varying from stallion to stallion. The air was then filled by a pump to create conditions closer to natural. AV was then allowed to equilibriate to temp. of 44-46°C. Though the stallions are not sensitive to temp. as some other species, yet full attention was given to temp. as the semen may be damaged at a temp. beyond 50°C. When the AV and stallion were ready, thorough lubrication of inner side of combination liner was done with sterile KY Jelly. Care was taken not to apply jelly in excess which may contaminate the semen. It was again ensured that the equilibrised tem. of prepared A.V. was between 44°C to 55°C.

Preparation of Stallion : Before collection, the stallion was taken out of stall and allowed to tease the mare until erection was attained. Some stallions attained erection while coming out of the stall and needed no teasing. The erected penis was grasped and washed with water at 40-42°C. Caution was taken not to irritate the penis. Soap was washed off from penis, as it is highly spermicidal.

Collection Technique : By the time stallion was prepared, the mare in heat was hobbled and her tail wrapped with tail bandage. A twitch was applied for proper control. As the stallion got ready, it mounted the mare. Semen collector remained on left side with the AV in his hand. On mounting when the stallion located the vulva, its penis was deflected into the AV which was kept at an angle of 30°. The collector at this moment had to lean towards the mare and pressed the AV against its thigh. With intromission, stallion begins thrusting movement, the pulsations of ejaculation could be felt at abdominal muscles, thigh muscles and at the base of the penis. In addition, the tail movements also gave indication of ejaculation. The free end of AV was lowered at this time so as to allow the semen to run into collection bottle. The handler placed his hand at the back of stallions knee to provide support for horse and protection for the collector.

As the stallion dismounted after ejaculation, the AV was removed while stripping the penis into AV. The valve assembly was opened, pressure released and half of the water

was removed from the jacket of AV. This allowed semen to flow freely into the collection bottle. The valve cap was again screwed and AV taken to the lab. Once the semen settled down, the collection bottle was removed and semen evaluated. Care was taken to keep the semen bottle in an incubator maintained at 38°C.

Semen Evaluation was done as per Singhvi (1989).

Dilution : Diluent was prepared as per Allen (1989) thus :

Skimmed Milk (Powder)	- 2.4 gm
Glucose D	- 4.9 gm
Streptomycine	0.1 gm
Crystallin penicillin	- 0.15gm
Double distilled water	- 100 ml

Fresh diluent was made, filtered and used every time. Dilution depended on the concentration of spermatozoa. Best result were achieved with dilution rate of 1:3.

Insemination process included the preparation of mare thus : (i) Mare was restrained in stocks (ii) Her tail bandaged and tied up to the left. (iii) The perineal region and vulval lips were washed with warm (40°C to 42°C) soapy water. (iv) Soap was removed by rinsing. (v) Area dried with absorbant paper napkins.

Well sterilised/autoclaved plastic sleeves, insemination cathetors (Pipette), syringes etc. were kept in an incubator at a temp. of 38°C. The diluted semen container was removed from incubator and correct volume of semen (providing 500 million sperms) required for insemination was aspirated in a syringe. Sterile sleeves were worn on one arm which was to be inserted in mare's vagina. A small amount of non spermicidal lubricant (preferably KY Jelly) was applied on the sleeve. The arm was then inserted in vagina and cervix located. It was ensured that atleast one finger could be inserted in the cervix. Now the sleeved hand was partially retracted and the tip of the insemination cathetor placed on the palm and carried to the tip of cervix. In mare the cervix is not equipped to serve as a reservoir for semen and hence the semen has to be deposited directly in uterus. The cathetor was therefore inserted in the uterus. Now the syringe containing semen was connected with the insemination cathetor and the piston pressed gently so that semen could be deposited in uterus. The cathetor was retracted slowly alongwith the arm. Care was taken not to permit air getting into vagina. Cold water was thrown on the back of mare immediately after insemination to prevent her urinating for some time.

Insemination was carried out only after ascertaining the ovulatory stage by rectal examination.

Once the insemination process was over, the tail bandage was removed and mare let loose. 5-8 mares could be inseminated by semen of one Stallion.

Results and Discussion

Results and conception/pregnancy based on rectal examination carried out after 60 days of insemination reveal that conception rate was very good in case of mulre breeding while it was fair in horse breeding (Table 1).

Conception rate was very high (57 to 67%) when insemination was done only once in first oestrus. Rate of conception significantly lowered when mares were inseminated in more than two oestrus (Table 2). It can be interpreted that mares inseminated into or more than two oestrus were carrying some

level of infection.

Advantages of AI in equines are : (i) Semen from one Stallion could be used for 4 to 8 mares, hence stallion power can be reduced significantly. (ii) Large number of mares can be inseminated in a very short time. (iii) Semen from each Stallion is used only after proper evaluation, increasing the chances of better conception. (iv) Many Stallions specially the donkey stallions and those of heavy draft breed, discharge the semen in mid vagina thereby reducing the chances of conception. In A.I. the evaluated semen is deposited directly in uterus increasing the chances of conception and (v) Mares with good height which could not be covered by normal stallions, could easily conceive by A.I.

The main disadvantage noticed in AI is that it makes the animal prone to certain infections. This can be overcome by proper autoclaving/sterilisation of equipment, using meticulous methodology and taking all precautions during insemination to contain the infection.

Acknowledgement

Author is thankful to Maj Gen K.L. Uthup, the Addl. DGRVS Army HQ for grant of permission to conduct A.I. work. Thanks are due to Col J.M. Rai, Commandant RTS and Depot Saharanpur for providing all the facilities and giving continuous inspiration for the work. Thanks are also due to all members of the Staff engaged in the process of collection, evaluation and insemination, without whose cooperation the work would not have been completed.

Table 1 : Over-all Conception Rate in Equines.

Class of animal	Number of mares inseminated	Mares found Pregnant	Conception Rate
HBBM	46	18	39.13%
MAMBBM	262	148	56.4%
GSMBBM	161	90	56.25%

Table 2 : Conception based on inseminations

Item	HBBM		MAMBBM		GSMBBM	
	No Insemi-nated	Found No Preg.	Inseminated	Found Pregn.	No Insemi-nated	Found Pregn.
Insemination in one oestrus	22	13 (59.09%)	136	86 (63.23%)	117	67 (57.26%)
Insemination in two oestrus	18	3 (16.66%)	98	45 (46%)	32	18 (55%)
Insemination in three or more oestrus	6	2 (33.33%)	28	10 (36%)	11	5 (46%)

REFERENCES

Anon (1978). Vet. Rec. **102** (24) : 515.

Allen RWR, Bowen J.M., Frank C.J., Jaffcott L.B. and Rossdale P.D.(1976) Equine Vet. J. **8**(2):72

Allen W.R. (1989) - Personal communication

Merkt H. (1984) Animal Breeding Abstr. **53** (9) : 5567

Muller Z. (1987) J. Reprod. Eertil. Suppl. : 35

Petalikova J. Metausek V and Muller Z. (1977) Animal Breeding Abstr. **46** (7):3162.

Singhvi N.M. (1989) J Rem. Vet. Corp. XXVIII (4)

IJAR:11:2:104-108:1990

Effect Of Prostaglandins On Buffalo Spermatozoal Migration In Cervical Mucus (In Vitro Studies)

E. MURALI NATH, B.B. REDDY, A.S.N. MURTHY, K.K. REDDY, and A.V. NARASIMHA RAO[1].

College of Veterinary Science, A.P.A.U., Tirupati-517 502 (A.P.)

ABSTRACT

The effect of addition of prostaglandins (PGs) E_2 and F_2 alpha at two different concentrations on *in vitro* cervical mucus penetration characteristics of Frozen Murrah buffalo semen was assessed. The PG treatment did not show any cognisable effect either on sperm penetration distance (SPD) or penetrated sperm count (PSC), when tested independently after 10 and 30 mts of initial semen mucus contact at 37°C. But sperm progression speed (SPS) after 10 mts of incubation was significantly ($P<0.01$) increased by the addition of PGs, the lower concentration being more effective. However, individual variation in the bulls was also significant for all the parameters tested.

• • •

In species like cattle, buffalo, sheep, goat etc. the cervix acts as the first barrier for sperm during its transport to the site of fertilization. The capacity of sperm to penetrate the cervical mucus is essential as the semen is deposited in vagina during natural mating or in the mid cervix during A.I. The cervical mucus penetration capacity of the spermatozoa has a positive correlation with the fertilizing ability in human beings (Reichman *et.al.* 1973; Insler *et.al,* 1979). An increase in sperm number in oviducts following A.I. with PG supplemented semen was found in rabbit and Sheep (Mandl, 1972; Chang *et.al.*, 1973; Edqvist *et.al.*, 1975 and Gustafsson *et.al.*, 1977). However, it is not clear whether this improved sperm migration across the cervix was caused by increased motility of the cervix or spermatozoa. Hence, the present study was undertaken to assess Murrah buffalo bull sperm migration characteristics within the cervical mucus (*in vitro*) by using frozen-thawed semen with or without the addition of PGs.

1. Asst. Director (A.H), Visakha Co-operative Dairy, Visakhapatnam (A.P.).

Materials And Methods

Six fertile Murrah buffalo bulls aged between 4 to 6 years and cycling she-buffaloes of different age groups were utilized for this study. Semen was collected twice a week for a period of 3 weeks by A.V. and evaluated. The semen sample suitable for insemination was extended in Tris egg-yolk citrate to contain 20 million spermatozoa per ml. which was subsequently partitioned into 3 aliquots. The PGs (M/S. Fuji Chemical Co., Tokyo, Japan) E_2 and F_2 alpha were added at a concentration of 2.5 ng and 1.5 ng respectively per ml of extended semen to one aliquot (T-2). The concentration of PGs were doubled in the second aliquot (T-3), the third being left without any addition (T-1). Subsequently the semen was frozen by rapid horizontal vapour-freezing technique in medium French straws, stored in liquid nitrogen until tested on cervical mucus.

The pooled cervical mucus which was collected during mid-estrus was stored at 4°C until used for sperm penetration tests. The cervical mucus penetration test was conducted as per the method described by Kremer (1965), with slight modification. The mucus filled capillary tube along with semen reservoir, containing frozen thawed semen was incubated at 37°C in vertical position (Fig 1) for a period of 10 mts or 30 mts to study either SPS, SPD or PSC.

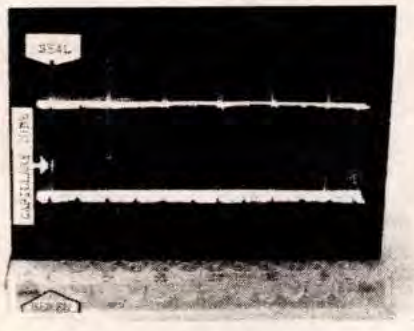


Fig.1 : Assembly for studying buffalo sperm migration in buffalo cervical mucus.

SPS was the average time taken by the three forward spermatozoa to travel a fixed distance of 140 microns in the mucus column. The SPD was the distance in cms from the open end of capillary tube, travelled by the progressive spermatozoa by the end of incubation time. The total number of sperm that could enter into the capillary mucus by the end of incubation expressed as PSC, SPS, SPD & PSC were recorded using phase contrast microscope with bio-therm. Data were analysed as per the methods described in Snedecor and Cochran (1980).

Results And Discussion

The values of SPS, SPD and PSC after 10 and 30 mts of exposure are given in Table 1 to 4.

Sperm progression speed : The mean SPS in oestrual cervical mucus column after 10 mts of incubation was found to be higher in T-2 compared to that in T-3 and control in that order ($P<0.01$). The improved motility observed apparently indicates the beneficial effect of PGE_2 which was shown to stimulate the sperm motility (Schoenfeld *et.al.*, 1975). The small improvement in the sperm motility in T-3 might be due to higher PGF_2 alpha concentration (Cohen *et.al.*, 1977). But individual bulls differed significantly ($P<0.01$) in their SPS both after 10 and 30 mts of exposure. The treatment x bull interaction was significant ($P<0.01$) only after 10 mts. The variation among bulls in their speed may be due to variation in the initial concentration of PG present in their ejaculates. The speed observed in the present study is lower than that of cattle reported earlier (Tampion and Gibbon, 1962; Roark and Herman, 1950 and Moeller and Van Demark, 1955). The observed differences in the SPS of cattle and

buffaloes may be due to the variation between the swimming speeds of their spermatozoa (Gallen and Roux, 1948; Mahmoud, 1952).

Sperm penetration distance (SPD) : The sperm penetration distance after 10 and 30 mts exposure was not affected by PG treatments. But the bulls differed significantly ($P<0.01$) in their SPD values. The treatment x bull interaction was also significant ($P<0.01$) at only 30 mts. The lack of treatment effect observed in this study is in close agreement with the findings of Memon and Gustafsson (1984) who reported SPD values at 15 mts and 180 mts in sheep.

The SPD value 2.25 cm/10 mts of incubation observed in buffaloes of control group in present study, is slightly less that the SPD value of cattle (Roark and Herman, 1950 and Moeller and Van Demark, 1955), but higher than that of Sheep (Memon and Gustafsson, 1984) and human beings (Kesseru, 1973). Interestingly the SPD 2.35 cm observed in our study after 30 mts of incubation, is less than that of corresponding observations 5.1 cm observed in human beings (Kesseru, 1973) and 3.62 cm in cattle (Akhtar *et.al.*, 1980). Apparently the linearity between time and SPD of buffalo semen does not extend much beyond 10 mts of incubation.

Penetrated sperm count (PSC) : The lack of treatment effect on PSC observed in this experiment is in close agreement with the findings of Memon and Gustafsson (1984) in sheep. The significant ($P<0.01$) difference observed between bulls only after 10 mts of semen mucus contact may be due to the variation in their initial SPS irrespective of the treatments.

Acknowledgement

The authors are grateful to the ICAR, New Delhi for sanctioning an Adhoc Research Scheme entitled "Seminal Prostaglandins in sub-fertile Murrah buffalo bulls".

Table 1 : Mean ± S.E. values of SPS, SPD and PSC after 10 mts and 30 mts of initial semen mucus contact.

Time	Parameter evaluated	T-1 Control	T-2 PGE_2+PGF_2 alpha (Lower conc.)	T-3 PGE_2+PGF_2 alpha (Higher conc.)
10 M	SPS	4.22 ± 0.3[x]	3.44 ± 0.1[y]	3.83 ± 0.2[z]
	SPD	2.25 ± 0.2	1.92 ± 0.2	1.98 ± 0.2
	PSC	253.22 ± 22.5	264.64 ± 30.5	210.28 ± 0.2
30 M	SPS	4.64 ± 0.2	4.43 ± 0.2	5.19 ± 0.2
	SPD	2.35 ± 0.3	3.06 ± 0.3	2.66 ± 0.3
	PSC	399.64 ± 25.4	558.21 ± 76.2	441.88 ± 28.5

Note : The values bearing different superscripts differ significantly ($P<0.01$)

Table 2 : Annova for SPS, SPD and PSC after 10 mts and 30 mts for initial semen mucus contact

		10 MTS						30 MTS					
		SPS		SPD		PSC		SPS		SPD		PSC	
		MSS	F	MSS	F	MSS	F	MSS	F	MSS	F	MSS	F
Between Treatments	2	5.53	8.38**	1.13	1.06NS	29578.2	2.25NS	3.17	1.57NS	2.88	1.44NS	188461	2.16NS
Between Bulls	5	13.59	20.59**	6.12	5.72**	113278.34	8.62**	12.03	5.96**	12.36	8.13**	55520.8	0.63NS
Bull X Treatment	10	3.48	5.27**	1.27	1.19NS	24548.41	1.87NS	1.85	0.92NS	12.78	8.41**	137775.4	1.97NS
Error	90	0.66		1.07		13136.91		2.02		1.52		87786.1	
Total	107												

NS - Not Significant
** - Significant at 1% level (P<0.01)

Table 3 : The mean ± S.E. of SPS, SPD, and PSC of different Bulls.

	10 MTS			30 MTS		
Bull No.	**SPS**	**SPD**	**PSC**	**SPS**	**SPD**	**PSC**
45	**3.30** ± 0.2	2.67 ± 0.4	337.28 ± 45.4	5.68 ± 0.1	3.86 ± 0.3	513.61 ± 36.7
110	3.34 ± 0.2	2.61 ± 0.3	296.22 ± 30.3	3.47 ± 0.5	1.98 ± 0.5	430.78 ± 143.0
583	3.19 ± 0.2	2.42 ± 0.2	264.50 ± 16.7	4.39 ± 0.4	2.23 ± 0.3	383.17 ± 42.9
619	4.98 ± 0.4	1.41 ± 0.1	143.28 ± 7.0	5.01 ± 0.2	1.88 ± 0.1	452.56 ± 45.2
3996	4.92 ± 0.3	1.43 ± 0.1	149.11 ± 7.1	3.89 ± 0.5	2.17 ± 0.4	384.33 ± 64.4
MPT	3.24 ± 0.2	1.77 ± 0.4	265.89 ± 38.8	4.72 ± 0.2	3.32 ± 0.5	499.39 ± 34.8

Table 4 : Effect of different PG treatments on SPS (Mean ± S.E.) at 10 mts

Bull	T-1	T-2	T-3
45	3.26 ± 0.2^{ef}	2.89 ± 0.2^{f}	3.75 ± 0.3^{def}
110	3.33 ± 0.3^{ef}	3.13 ± 0.2^{f}	3.57 ± 0.3^{def}
583	3.02 ± 0.2^{f}	3.08 ± 0.1^{f}	3.48 ± 0.4^{def}
619	7.00 ± 0.4^{a}	4.13 ± 0.3^{cde}	3.80 ± 0.2^{def}
3996	5.54 ± 0.4^{b}	4.30 ± 0.5^{cd}	4.92 ± 0.4^{bc}
MPT	3.17 ± 0.3^{f}	3.09 ± 0.2^{f}	3.46 ± 0.9^{def}
Overall treatment	4.22 ± 0.3^{3}	3.44 ± 0.1^{1}	3.83 ± 0.2^{2}

Note : The values bearing different superscripts differ significantly ($P<0.01$)

REFERENCES

Akhtar, K.K., Singh, K.K., and Sinha, R.P. (1980) Studies on migration rate of spermatozoa in bovine cervical mucus Indian Vet. J. **57** : 386-390.

Chang, M.D., Hund, D.M., and Polge, C. (1973) Effects of PGs on sperm and egg transport in the rabbit. Advanced Biosciences, **9** : 805-810.

Cohen, M.S., Colin, M.J., Golimbu, M. and Hotchkiss, R.S. (1977) The effect of PGs on sperm motility. Fertil. and Steril. **28**(1) : 78-85.

Edqvist, S., Einarsson, S. and Gustafsson, B. (1975) Effect of prostaglandin F_2 alpha on sperm transport in the reproductive tract of the ewe. Acta Veterinaria Scandinavica, **16** : 149-151.

Gallein, L. and Roux, P. (1948) L'insemination artificiale chezles animaux domestiques. Paris. Resses. Universitaires de France, 226 PP.

Gustafsson, B.K., Graham, E.F., Crabo, B.G., Pavelko, M.K. and Wagner, W.C. (1977) Prefreeze supplementation of ram semen with PGE_1 and PGF_2 alpha. Effect on sperm vitality *in vitro* and on the sperm transport in the ewe. Proc. 10th Annual Meeting. Society of Reproduction, August, 14-17.

Insler, V., Berstein, D., Glezerma, M. and Misqav, N. (1979) Correlation of seminal fluid analysis with mucus-penetration ability of spermatozoa. Fertil. and Steril. **32** : 316-319.

Kesseru, E. (1973). *In Vivo* sperm penetration and *in vitro* sperm migration tests. Fertil. and Steril. **24** : 584-591.

Kremer, J. (1965). A simple sperm penetration test. International Journal of Fertility, **10**(3):209-215.

Mahmoud, I.N., (1952). Some characteristics of the semen of Egyption buffaloes. Bull. Fac. Agric. Fouad I University (Cairo) No. 15 : 16. Animal Breeding Abstract, **21**:No.1262.

Mandl, J.P. (1972). The effect of prostanglandin E_1 on rabbit sperm transport *in vivo*. J. Repord. Fertil., **31**:263-269.

Memon, M.A. and Gustafsson, B.K. (1984). Effects of various concentrations and combinations of PGs on *in vitro* migration of frozen thawed ram spermatozoa in cervical mucus of ewes. Theriogenology, **21**:841-48.

Moller, A.N., and Van Demark, N.S. (1955). Fertility and Sterility, **6**:506.

Rechman, J., Insler, V. and Serr, D.M. (1973). A modified *in vitro* spermatozoal penetration test. II. Application to fertility investigations. International Journal of Fertility, **18**: 241-45.

Roark, D.B. and Herman, H.A. (1950). Animal Breeding Abstract, **19**:145.

Schoenfeld, C., Amelar, R.D. and Dubin, L. (1975). Stimulation of ejaculated human spermatozoa by caffine. Fertility and Sterility, **126**:158-61.

Snedecor, G.W. and Cochran, W.G. (1980). Statistical Methods. 7th Edn. Lowa State University Press, Ames, USA.

Tampion, D. and Gibbon, R.A. (1962). Swimming rate of bull sperm. Nature, Lond., **194**:695.

IJAR:11:2:109-110:1990

Semen Studies During Vaccination Stress In Jersey Bulls

P. S. GAHLOT, B. L. BISHNOI, and I. S. KOHLI

Department of Obstetrics and Gynaecology, College of Veterinary and Animal Science, Bikaner-334 001.

ABSTRACT

No marked difference between bacterial and viral vaccination, on bull semen pictures, was recorded. However varied individual response of bulls to individual vaccines was observed.

* * *

The bulls located at the artificial insemination centres are vaccinated annually against bacterial and viral diseases as a matter of routine. As such it was considered desirable to ascertain the comparative effects of prophylactic vaccination on semen quality of breeding bulls.

Narasimhan *et at* (1970) and Venkataswami and Rao (1970) reported that Rinderpest (RP) and Foot and Mouth disease (FMD) vaccination produced high percentage of sperm abnormalities. Latal Niedzielska (1971) and Rao and Venkataswami (1971) found increased sperm abnormality post-FMD vaccination. Venkataswami *et al* (1972) observed that viral vaccination produced more deleterious effects on semen quality of bulls than bacterial vaccination.

Materials And Methods

Five Jersey bulls (M-76, I-11, X-378, X-388 and CJ) aged 5 to 6 years, were used for this study. All the bulls were maintained under uniform conditions of management and feeding and were clinically normal. They were subjected to pre-experimental trial and their semen was evaluated for total ejaculate, initial motility, hydrogen ion concentration (pH), sperm concentration, live and dead percentage and abnormal sperm percentage.

During the year, all the five bulls were vaccinated at different intervals against black quarter, haemorrhagic septicemia, Rinderpest and foot and mouth disease vaccines. Pyrexia resulting due to FMD vaccination was controlled by giving 30 ml Novalgin I.V.

All the bulls showed individual response to rise in temperature. The adverse effect on semen quality was mild and for a short duration.

Out of 4 bulls studied, the average sperm abnormalities recorded were M-76 (10.66%), X-388 (14%), I-11 (8%) and CJ (22.2%) compared to Venkataswami *et. al.* (1972) who reported 20.8 percent. This showed individual variations.

H.S. Vaccination : The study showed that : (i) Vaccination had an adverse effect on the semen quality but effect was mild and temporary and semen quality returned to normal in short time. (ii) Post vaccination pyrexia, which varied from bull to bull, was not necessary for causing deterioration in semen quality, and (iii) Vaccination had no uniform effect on the various semen characteristics. In some bulls some characteristics were affected while in other bulls other characteristics were affected.

However, the effect of vaccination on bull CJ was most severe. Apart from registering very high rise of temperature (106°F),

the bull did not give any ejaculate for 3 weeks and all the parameters except for pH were adversely affected till 6 weeks, post-vaccination.

Comparison of the effect of Bacterial and viral vaccination on Post-vaccination semen picture : No marked difference between bacterial and viral vaccination on semen picture, was recorded. However, individual response of bulls to individual vaccines was observed. Some bulls showed very severe reaction to BQ and HS vaccines than others. Intensity of post-vaccination pyrexia which varied from bull to bull was not necessary for causing deterioration in the semen quality.

Bull CJ showed very high rise of temperature after vaccination with BQ and H.S. vaccines, while no such post vaccination effect after bacterial vaccination has been reported earlier.

In FMD vaccination, previous workers did not report any rise in post-vaccination temperature whereas in the present study we recorded much difference. In bull No. X-378, there was high rise of temperature (104°F) due to vaccination, whereas in bull M-76 the temperature due to vaccination was controlled. Both these bulls showed similar degree of adverse effects.

So far as Rinderpest vaccination is concerned previous workers mostly reported very high rise of post-vaccination temperature up to 105° — 108.6°F, except Venkataswami *et.al.* (1972). They reported post-vaccination pyrexia by G.T.V. vaccine but in the present study no post-vaccination pyrexia was noticed. A mild adverse effect on the semen picture was noticed in comparison to the findings of previous workers.

REFERENCES

Latala-Niedzielska, M. (1971). Polskie Archium Veterynaryjna, 14:211-217. Cf.Anim. Breed. Abstr. 42:40.

Narasimhan, K.S., Krishnan, A.R. and Syed Abdul Quayam (1970). Indian Vet. J. 47:19-22.

Venkataswami, V. and Rao Jagannadha, V. (1970). Indian Vet. J. 47:23-29.

Venkataswami, V.; Pattabiraman, S.R.; Daniel, J. and Sunderavadanan, V.K. (1972). Indian Vet. J. 49:1012-1016.

IJAR:11:2:110-112:1990

Incidence Of Leptospirosis In Breeding Bulls

B. RAMESHKUMAR[1], R. C. RAJASUNDARAM[2] and E. KANDAVEL

Livestock Research and Development Centre, Erode-638 003 Tamil Nadu

Leptospirosis in cattle causes abortion, haemoglobinuria, anaemia and in some cases neurological disorders. Rajasundaram and Nadunchelliyan (1988) reported Leptospiral abortions in cattle in Erode District of Tamil Nadu. There is paucity of literature about the incidence in breeding bulls. In the present study a survey was conducted at Government Bull stations situated around

Present Address :

1. Assistant Professor, Department of Clinics, MadrasVeterinary College, Madras-600 007.
2. Assistant Professor, Poultry Research and Development Centre, Tirupur-638 601.

Erode town of Tamil Nadu to find out the incidence of Leptospirosis in breeding bulls.

Materials And Methods

The study was conducted on 64 breeding bulls (24 Zebu breeding bulls and 40 buffalo breeding bulls) stationed at six government bull stations. Paired blood serum samples from these animals were collected 2-3 weeks apart for serological testing by Microscopic Agglutination Test (MAT). Two animals which were showing clinical signs such as pyrexia, anaemia, dysponea and neurological signs were treated with parenteral penicillin (3 million units) and streptomycine (5 gms) twice daily for 5 days.

Postmortem examination was conducted on one animal which died inspite of the treatment. Liver, spleen and kidney tissue pieces were collected and preserved in 10% formal saline. Paraffin embedded tissue sections were cut and stained with Haematoxylin and Eosin and Levaditis stain for histopathological examination.

Results And Discussion

Out of 64 breeding bulls examined, two Jersey bulls showed a serum titre value of 1:320 for Leptospira pomona agglutinins by microscopic agglutination test (MAT). They showed clinical signs such as pyrexia (40-41°), anorexia, anaemia and loss of general condition. One animal showed arched back, stiffness of hind limbs and it died after a week inspite of the stiff antibiotic treatment with strepto-penicillin. Postmortem examination of the carcass showed marked nephrosis, pale and enlarged liver. The abdominal cavity was filled with ascitic fluid. There were some degenerative changes in the terminal parts of the spinal cord. Histo-pathological examination of the liver tissue showed focal necrosis around the central vein. Spleen and kidney tissues showed degenerative changes. Kidney sections stained by Levaditi's method showed spiral bodies resembling Leptospira organisms. The other affected animal responded well for the treatment with strepto-penicillin and the symptoms disappeared after 10 days. The paired serum titre also showed a decline in Leptospiral agglutinins after 3rd week.

In the present study, out of 64 breeding bulls, two animals (3.1%) showed the incidence of Leptospirosis. No incidence was noticed in buffalo breeding bulls while the incidence in zebu breeding bulls was 8.33%. Sleight and Williams (1961) reported that breeding bulls infected with L. pomona could infect susceptible cows through semen leading to abortion. Sleight (1965) advised that only breeding bulls free from Leptospirosis should be used for A.I. Hence, the infected animal was given sexual rest for one month and the semen from the recovered animal was put into use only after the paired serum titre value for Leptospirosis had come down.

The postmortem lesions recorded from the animal which died after a week were similar to those reported by Rajasundaram and Nedunchelliyan (1988). Treatment with penicillin-streptomycin at the rate of 3 million units and 5 gms respectively was found quite satisfactory in controlling the Leptospiral infection as reported by Roberts (1971). However, one animal died inspite of the treatment due to the severity of the disease. Microscopic agglutination test (MAT) was found helpful in confirmatory diagnosis of Leptospirosis by examination of paired serum within 2-3 weeks interval as suggested by Roberts (1971).

Since the infected and carrier bulls could shed the organisms by urine and semen and possibly infect the susceptible female animals, a routine serological test to rule out leptospirosis in breeding bulls is advisable in all established breeding bull stations.

Acknowledgements

The authors are thankful to the Professor and Head Department of Preventive Medicine, Madras Veterinary College for the help rendered in conducting serological tests and the Director, Centre for Animal Health Studies, Madras Veterinary College for his valuable suggestions and encouragement.

REFERENCES

Rajasundaram, R.C. and Nedunchelliyan, S. (1988). Leptospiral abortion in cattle and buffaloes. Indian J. Anim. Reprod. 9(2):104-105.

Robert, S.J. (1971) Veterinary obstetrics and genital disease. 2nd Edn. CBS Publishers and Distributors.

Sleight, S.D. (1965) The role of Penicillin and Streptomycin in the prevention of transmission of bovine leptospirosis by artificial insemination. Amer. J. Vet. Res. 26(111):365.

Sleight, S.D. and Williams, J.A. (1961). Transmission of bovine leptospirosis by Coition and artificial insemination (A preliminary report) JAVMA. 138(3):151.

IJAR:11:2:112-113:1990

Biometry Of Non-Pregnant Genitalia Of African Zebu Cattle

F. S. CHAUHAN* and ALHAJI Y. ADAMU

Department of Veterinary Surgery and Reproduction, University of Maiduguri, Maiduguri, Nigeria.

On perusal of the available literature it was observed that not much information is available on biometry of genital organs of non-pregnant zebu cattle (Dobson and Kamonpatana, 1986). Therefore, this study was undertaken to establish the norms of reproductive organs for zebu cattle.

The study was conducted on 45 non-pregnant genital organs of heifers and cows obtained from abattoir, Maiduguri, Borno State. The animals were of different breeds (Bhunaji, White Fulani, Wadara and Kuri) age, and live weights. The dimensions were taken as per Edward (1965).

The weight (Mean ± SE) of genitalia of heifers and cows was 0.43 ± 0.03 and 0.79 ± 0.02 Kg, respectively. The mean length, breadth, thickness and weight of left ovary in heifers were 2.50 ± 0.06, 1.44 ± 0.05, 0.84 ± 0.15 cm, 3.80 ± 0.12 gms, respectively. The corresponding values for left ovary of cows were 2.81 ± 0.02, 1.56 ± 1.01, 1.04 ± 0.01 cm and 4.48 gms, respectively. The length, breadth, thickness and weight of right ovary in heifers and cows were 2.53 ± 0.05 and 2.84 ± 0.12, 1.45 ± 0.04 and 1.63 ± 0.02, 0.85 ± 0.4 and 1.05 ± 0.01 cm, 3.53 ± 0.10 and 5.48 ± 0.04 gms, respectively.

* Present Address : Associate Professor, Deptt. of Veterinary Obstetrics and Gynaecology, Punjab Agricultural University, Ludhiana-141004 (Punjab, India).

Students' 't' test revealed that ovaries of heifers were smaller compared to cows ($P<0.05$).

The mean length of left oviduct of heifers and cows was 18.10 ± 0.15 and 19.46 ± 0.05 cm, respectively. The corresponding values for right oviduct were 17.70 ± 0.16 and 20.38 ± 0.05 cm, respectively. The difference in length of oviducts between heifers and cows was significant ($P<0.05$)

The mean length and diameter of left Uterine horn of heifers were 18.10 ± 0.15 and 2.44 ± 0.06 cm, respectively.

The corresponding values for cows were 19.46 ± 0.05 and 2.89 ± 0.03 cm, respectively. The mean length and diameter of right horn of heifers were 17.70 ± 0.16 and 2.67 ± 0.06 cm respectively. The corresponding values for cows were 30.21 ± 0.07 and 2.89 ± 0.03 cm, respectively. Student's 't' test revealed that the difference in length and diameter of horns between heifers and cows was highly significant ($P<0.01$). The mean length and diameter of body of uterus in heifers were 2.10 ± 0.07 and 3.23 ± 0.08 cm, respectively. The corresponding values for body of uterus of cows were 2.27 ± 0.03 and 3.24 ± 6.03 cm, respectively. The mean length and diameter of cervix of heifers were 7.38 ± 0.10 and 3.18 ± 0.07 cm, respectively. The corresponding values for cows were 8.08 ± 0.04 and 3.48 ± 0.02 cm, respectively. The difference in length and diameter of cervix in heifers and cows was highly significant ($P<0.01$).

The average size of ovaries observed in this study closely resembles with earlier report of Nigerian Cattle (Herbert, 1974). However, Lamorde and Kumar (1978) reported that mean width and length of ovaries were 2.7 ± 0.70 and 1.8 ± 0.6 cm, respectively, which is contrary to these findings.

A comparison of dimensions of genitalia shows that zebu-cattle genitalia were smaller compared to European breeds of cattle (Edward, 1965).

REFERENCES

Dobson, H. and Kamonpatna, M (1986) : A review of female cattle reproduction with special reference to a comparison between buffaloes, cows and zebu J. Report. Fet. 77:1-36.

Edward, M.J. (1965) : Observations on the anotomy of reproductive organs of cows with special reference to those features sought during examination per rectum. Newzealand Vet. J. 13:25-37.

Herbert, W.J. (1974). The reproductive history of cows slaughtered in North West Nigeria as indicated by examination of their ovaries. J. Tropical Animal Health and Prod. 6:223-229.

Lamorde, A.G. and Kumar, M.S.A. (1978): Observations on the ovaries of Zebu cattle in Northern Nigeria. J. Research in Vet. Sci. 24:305-307.

IJAR:11:2:114-116:1990

'On Farm' Embryo Transfer in Crossbred Cows Under Indian Field Conditions

A. SUBRAMANIAM, K.P. DEVARAJAN and M. MOHANAN

Embryo Transfer State Centre, The Periyar District Co-operative Milk Producers' Union Ltd. Vasavi College Post, Erode 638 316.

ABSTRACT

Observations of On farm non-surgical embryo transfer work are presented. 19 out of 31 donor cows responded to superovulatory treatment with PMSG. 45 embryos were recovered non-surgically, of which, only 24 embryos were found fit for transfer. Of the 24 embryos transferred in naturally synchronised recipients, only 7 resulted in pregnancy. Two animals aborted after 120 days. Five calves (3 female and 2 male) were born unaided. The recovery rate of embryo per donor cow was 2.36, whereas pregnancy and calving percentage was 29.16 and 20.08, respectively.

* * *

The advent of non-surgical trans-cervical embryo recovery and transfer has simplified the methods of embryo transfer and all its benefits to the doorstep of the farmer. This process is generally known as "On farm or hands on" embryo transfer.

An attempt has been made in this paper to share the experiences of embryo transfer work from the first On farm embryo transfer facility established in the country at Erode.

Materials And Methods

Thirty one apparently healthy, lactating and cycling crossbred cows belonging to members of various Primary Milk Producers' Co-operatives were selected as donors for On farm embryo transfer work during 1986-1988. The criteria for selection were based on the proximity of location of the farm to the embryo transfer facility, owner's ability to co-operate with the programme, milk yield and availability of recipients.

Upon selection, the donor cows were observed for two consecutive estrous cycles to establish the normalcy of the cycle. On day 9 of the third estrous cycle, the donor cows were injected with PMSG (Folligon, Inter Vet., Holland). The dose regime adopted was 1800 I.U. for Jersey crossbreds and 2000 I.U. for Friesian crosses as suggested by Schams *et.al.* (1978) and Heath (1984). Forty eight hours following administration of PMSG, each donor cow was injected with 25 mg of prostaglandin F2 alpha i/m (Glandin N. TAD Pharmazeutisches Werk GMBH, West Germany and Dinofertin, Alved, India depending on availability). Following PG treatment, donor cows were monitered for estrus. Donor cows exhibiting estrus were inseminated thrice at 12 hourly interval with progeny tested 0.25 cc NRF semen. Embryo flushings were carried out on day 6, following first AI.

Procedures of Newcomb (1982) were adopted for trans-cervical embryo recovery, using 2 way round tip Foley's catheter positioned by inflating the cuff at the level of greater curvature of the horn to be flushed.

Flushings were done by passing Dulbecco's phosphate buffered saline (PBS) containing 2% foetal Calf serum into the horn through the catheter and the same was recovered by intermittent gravity flow along teflon rubber tubing into 1000 ml graduated flush fluid holding bottle (Duran, Germany) as described by Moore (1984). Embryos were rinsed out by passing 50 ml of PBS at a time and at the same time by applying gentle massage to the uterine horn to facilitate easy flushing. The flow of PBS in and out of the uterus was controlled by clamps. Upto 500 ml of PBS was used for flushing each horn.

Final flush fluid from each donor cow was allowed to settle for 20 minutes and its volume reduced to 20-30 ml with the help of 50 μ plankton sieve mounted on a disposable plastic syringe to concentrate embryos (Moore, 1984). After reduction of volume, the syringe mounted filter surface was carefully washed through a jet of 15 ml PBS into a 100 x 15 mm grid petridish (1012 Integrid, Falcon, USA) into which embryo concentrated medium was also drained. The inner surface to the holding bottle was rinsed thrice with 5 ml PBS at a time and poured into grid petridish to avoid loss of embryos adhering to its surface. The final volume in the grid petridish was around 70 ml. The same was examined under stereomicroscope (Labo, India) at 40X magnification. The light source of the microscope was modified with ordinary torch illumination unit to prevent warm light as well as power disruptions.

When embryos were located, they were transferred to small petridish (35 x 10 mm Falcon, USA) containing PBS with FCS, using unopipette attached to a tuberculin syringe. Embryos from holding petridish were graded adopting methods described by Shea (1981). Morphologically sound embryos were transferred non-surgically to the recipients with naturally synchronized estrous cycle to donors using a 0.25 ml AI straws (IMV, France) as described by Elsden and Seidel Jr (1984). At day 60 post transfer, pregnancy diagnosis was done in the recipients by rectal palpation.

Results And Discussion

19 out of 31 cows responded to superovulation. The response was lower than that reported by Schams *et.al.* (1978) and Newcomb (1982). However, Miller (1984) has reported large variations in donor response to PMSG treatment. Further, Seidal Jr and Seidal (1981) and Heath (1984) have stated that some donor cows respond with superovulation for PMSG treatment but do not exhibit estrus signs. Since exhibition of signs of estrus was mainly relied upon in the present work, few donor cows might have been left out resulting in low rate of superovulatory response.

Average embryo recovery per donor was 2.36 (Table 1) with a range of 0-7 embryos. For On farm embryo recoveries, Elsden and Seidel Jr. (1984) have reported 2.68 embryos as mean recovery from superovulated cows with unknown reproductive histories. Initially on five occasions virtually no embryos were recovered and on one occasion by the time flushing was completed and embryo search was on it was twilight time and swarms of insects attracted by microscopic illumination forced us to abandon the work resulting in loss of four embryos but gain in valuable knowledge that on farm flushing should never be attempted in the evenings.

In this study a total of 45 embryos were recovered, of which only 24 were of transferable quality. Such embryos were implanted

into the ipsilateral horn of naturally synchronized recipients having palpable corpus luteum. Seven pregnancies were recorded, of which two ended in abortion at 124 and 136 days, respectively, resulting in five unaided calvings. First calf born was a male - the first of its kind born out of On farm embryo transfer in the country. Subsequently, one male and three female calves were born.

Pregnancy and calving rates were 29.16% and 20.08% respectively. These rates are lower than the observations of Elsden (1980), Seidel Jr and Seidel (1981), Newcomb (1982) and Peeples (1984). Furthermore, natural synchrony of recipients and unknown reproductive history of donor cows, tends to lower success rate. It may thus be concluded that Owner's ability to co-operate with the programme, distance, donor's response, recipient synchrony and facilities of ET Centre appear to be the major contributory factors for success rate of On farm embryo transfer programmes.

Table 1 : Superovulation, embryo recovery and results of On farm embryo transfer in crossbred cows.

Sr.No.	Description	Numbers
1	Donor Cows superovulated	31
2	Donor Cows responded	19
3	Donor Cows flushed	19
4	Embryos recovered	45
5	Embryos abandoned	4
6	Embryos transferable	24
7	Embryos transferred	24
8	No.of recipients pregnant	7
9	No.of recipients calved	5
10	Sex of calves - Male	2
	Female	3
11	No. of abortions	2
12	% of pregnancy	29.16
13	% of calving	20.08
14	Average embryos/flush	2.36

REFERENCES

Elsden, R.P. (1980) Bovine Embryo Transfer. Proc. Soc. Theriogenology 14:101-133.

Elsden, R.P. and Seidel, G.E. Jr (1984) Embryo transfer procedures for Cattle. Proc. No. 70 Bovine Embryo Transfer workshop, University of Sydney, Australia, pp. 51-95.

Heath, T.D. (1984) Superovulation, Breed comparisons and Drug Comparisons. Proc. No. 70 Bovine Embryo Transfer workshop. University of Sydney, Australia, pp. 39-41.

Moore, R.L. (1984) Non-surgical collection of Bovine Embryos. Proc. No. 70 Bovine Embryo Transfer workshop. University of Sydney, Australia, pp. 205-208.

Newcomb, R. (1982) Egg recovery and transfer in Cattle. Mammalian Egg transfer (Ed. C.E. Adams) CRC Press Bocaraton, Florida, USA.

Peeples, J.G. (1984) Advantages and disadvantages of On the farm Embryo transfer. Proc. NAAB and IETS. Ann. Conf. Artific Insem. and embryo transfer in Beef Cattle, pp. 36-37.

Schams, D., Menzer, L., Schallen Berger, E., Hoffman, B., Hahn, J., and Hahn, R. (1978) Some studies on pregnant mare serum gonadotrophin (PMSG) and endocrine responses after application for superovulation in Cattle. Control of reproduction in the Cow. Comm. European. Ecom. Commum. Luxemberg. pp. 122-143.

Seidal, G.E. Jr. and S.M. Seidel (1981) Embryo transfer industry New Technologies in Animal breeding (Ed. B.G. Bracket, G.E. Seidel, Jr. and S.M. Seidal) Academic Press, New York, USA, pp 41-88.

Shea, B.P. (1981) Evaluating Bovine embryo. Theriogenology 15(1): 31-49.

IJAR:11:2:117-120:1990

Haematological And Blood Bio-Chemical Profiles Of Fertile And Non-fertile Estruses in Kankrej Heifers*

B. V. GUJAR[1], A. LATIF[2], V. P. VADODARIA[3], and K. P. SHUKLA[4].
College of Veterminary Science and Animal Husbandry, Sardar Krushinagar-385 506 Gujarat.

ABSTRACT

Haematological (Hb and PCV) and biochemical (blood glucose and total serum proteins) attributes were studied in 36 Kankrej heifers which were divided into two groups of fertile and non-fertile estrus. Age and body weight did not differ significantly between two groups. Levels of both Hb (11.32 ± 0.21 g %) and PCV (38.35 ± 0.71 %) were higher in fertile estrus group but the difference was significant (P≤0.01) for Hb only. Non-fertile estruses were associated with significantly higher (P≤0.01) level of blood glucose (78.7 ± 1.9 mg %) and lower level of serum proteins (6.34 ± 0.12 g %). The correlations between age and body weight in both groups and blood glucose and body weight in the non-fertile group were positive and highly significant.

Haematology and biochemical constitutents of blood have great diagnostic value in the clinical practice. They also aid in understanding the reasons associated with low calf crop in most of the domestic animals (Mc Clure, 1965, Lamond, 1970; Awasthi and Kharche, 1987). Keeping these in view, an attempt was made to evaluate the difference, if any, in the haematological and blood biochemical profiles of fertile and non-fertile estruses in Kankrej heifers.

Materials And Methods

Haematological and blood biochemical profiles of 36 Kankrej heifers, stationed at Live-stock Research Station, GAU, Sardar Krushinagar, were studied on the day of estrus. The heifers which did not conceive formed the non-fertile estrus group, while those conceived at first or subsequent estrus formed the fertile estrus group. The heifers were reared under standard managemental practices and subjected to thorough gynaecological examination for the detection of genital abnormalities and infection, if any.

Blood samples were analysed for Hb and PCV (Schalm *et.al.*, 1975), blood glucose (Oser, 1979) and total serum proteins (Wooton and Freeman, 1982). For estimation of glucose, blood samples were collected using sodium fluoride as an anticoagulant.

Results And Discussion

The age of heifers at fertile estrus was 915.0 ± 23.0 days with an average body weight of 306.7 ± 4.9 kg. (Table 1) The corresponding values in case of heifers having non-fertile estruses were

* Part of Ph.D. Thesis of senior author submitted to Gujarat Agricultural University.

1. Subject Matter Specialist (Animal Production) O.R.P., Ambajogai-431 517 (M.S.)
2. Assistant Professor (Physiology)
3. Professor of Physiology
4. Professor of Livestock Production and Management.

854.0 ± 29.0 days and 294.6 ± 7.2 kg, difference between groups being non-significant (Table 2) indicating that age and body weight were not associated with fertile and non-fertile estruses.

Level of Hb in the fertile estrus group (11.32 ± 0.21 g%) was significantly (P≤0.01) higher than that of non-fertile estruses (1041 ± 0.20 g %). Higher Hb content in fertile estruses was found associated with higher PCV values. Significantly higher haemoglobin concentration in fertile estrus group might be due to the fact that the physiological functions of various body tissues including the reproductive tissues, depended upon the availability of energy from the oxido-reductive reaction, which otherwise were directly dependent on the supply of oxygen. The lower levels of Hb and PCV at non-fertile estruses as recorded in the present investigation were in agreement with those of Sharma *et.al.* (1983) and Kumar *et.al.* (1985, 1986).

The blood glucose concentration at fertile estruses (57.20 ± 1.90 mg%) was significantly lower (P≤0.01) than at non-fertile estruses (78.70 ± 1.90 mg %). Mc Clure (1965) observed that variation in blood glucose was closely linked with estrous cyclicity and fertility. The present trend was partially in accordance with that of Rao *et.al.* (1981) who observed that the glucose concentration in repeat breeders (43.24 ± 2.87 mg %) was higher than that in the normal cyclic heifers (42.23 ± 1.35 mg %). Awasthi and Kharche (1987) obtained non-significantly higher blood glucose values in fertile than infertile crossbred cows. Higher glucose level at non-fertile estruses was in agreement with Parmar *et.al.* (1986) who indicated that the specific metabolic stress might have elevated blood glucose level in repeat breeders. From the present findings of higher blood glucose and lower Hb content in the non-fertile group it could be inferred that there might be inadequate utilization of glucose by the tissues of reproductive tract due to the change in absorption and catabolism rate caused by specific hormone status leading to failure of fertilization.

The mean serum protein concentration at fertile and non-fertile estruses was 7.59 ± 0.09 and 6.34 ± 0.12 g%, respectively. This difference was highly significant (P≤0.01). These values are in line with those indicated by Pyne and Maitra (1980) for various Zebu cattle. Kavani *et.al.* (1987) emphasized that low level of serum proteins influenced that reproductive status in Kankrej heifers. The lower level of total serum proteins might have caused deficiency of particular amino-acids required for the synthesis of various releasing hormones and pituitary hormones causing in turn reproductive disturbances. Low levels of serum proteins at non-fertile estruses might be one of the factors associated with failure of fertilization.

Only the age and body weight were found dependent significantly (P≤0.01) in both groups. The coefficients were 0.415 and 0.642 at fertile and non-fertile estruses, respectively (Table 3). Contrary to this, the blood profile appears mostly independent of age and weight except the blood glucose concentration. In case of non-fertile estruses the glucose concentration was positively correlated with the body weight (P≤0.01) which may be due to individual variations between the animals within the same group.

Acknowledgement

The authors are thankful to Dr. N.S. Radadia, Research Scientist, LRS, for providing necessary facilities. Thanks are also due to Dr. F.S. Kavani, Associate Professor of Gynaecology, for carrying out gynaecological examination of experimental animals.

Table 1 : Mean values for Age, weight and haematological and biochemical status at fertile and non-fertile estruses in Kankrej heifers.

Heifer group	Age (days)	Bodyweight (kg)	Hb (g%)	PCV (%)	Glucose (mg %)	Serum proteins (g %)
Fertile (n-34)	915.0±23.0	306.7±4.9	11.32±0.21^{b}	38.35±0.71	57.20±1.90^{a}	7.59±0.09^{b}
Non-fertile (n-24)	854.0±29.0	294.6±7.2	10.41±0.20^{a}	36.33±0.98	78.7±1.90^{b}	6.34±0.12^{a}

Values with different superscripts in a column differ significantly.

Table 2 : Analysis of variance of age, weight, haematological and biochemical status at fertile and non-fertile estruses in Kankrej heifers.

Source of variation	d.f.	M.S.					
		Age	Body weight	Hb	PCV	Glucose	serum proteins
Between groups	1	2063.0	52,456.0	9.79**	60.63	6992.8**	21.80**
Within groups	56	998.0	19,074.0	1.19	22.09	96.7	0.33

* ($P \leq 0.05$); ** ($P \leq 0.01$)

Table 3 : Correlations between age, body weight and blood constituents at fertile and non-fertile estruses.

Characters	Age	Body weight	Hb	PCV	Glucose	Serum proteins
Age	-	0.415**	0.111	0.188	0.059	-0.016
Body weight	0.642**	-	0.131	-0.054	-0.071	0.049
Hb	0.080	0.170	-	0.311	0.332	-0.133
PCV	0.153	-0.046	0.276	-	0.228	0.044
Glucose	0.369	0.511**	0.048	0.006		0.210
Serum proteins	-0.039	0.210	0.042	0.228	0.148	-

1. Values above diagonal are for fertile estruses (n-34) and below diagonal are for non-fertile estruses (n-24)

REFERENCES

Awasthi, M.K. and Kharche, K.G. (1987). Studies on some constituents in normal cycling, fertile and infertile repeat breeder crossbred cows. Indian J.Anim.Reprod. 8:95-97.

Kavani, F.S., Sharma, V.K., Siddiquee, G.M. and Vadodaria, V.P. (1987) Serum proteins, ascorbic acid and total cholesteroal in anoestrus Kankrej heifers. Indian J.Anim.Reprod. 8:148-150.

Kumar, S., Sharma, M.C., Agrawal, S.K. and Dwivedi, S.K. (1985). Haematological changes in normal cyclic, anoestrus and repeat breeding cattle. Indian Vet. med. J., 9:234-235.

Kumar, S., Sharma, M.C. and Dwivedi, S.K. (1986). Blood cellular "changes" in non-descript bovine heifers during anoestrus and repeat breeding condition. Indian J.Dairy Sci., 39:459-460.

Mc Clure, T.J. (1965). A nutritional cause of low non-return rate in dairy herds. Aust.Vet.J., 41:119-122.

Oser, B.L. (1979) Hawk's Physiological Chemistry, 14th Edn. Tata Mc Graw Hill Publ. Co. Ltd., New Delhi.

Parmar, K.S., Mehta, V.M. and Patel, J.M. (1986) Biochemical profiles of repeat breeding cross-bred cattle in relation to different stages of oestrous cycle. Indian J.Anim.Reprod. 7:31-35.

Pyne, A.K. and Maitra, D.N. (1980). The Biochemical constituents of blood of Hariana, Sahiwal and Gir heifers, Indian J.Dairy Sci., 33:397-398.

Rao, D.G., Ambaprasad, A.B., Jayaramakrishna, and Rao, S. (1981). Studies on some biochemical constituents of blood in Ongole cows. Indian Vet.J., 58:870-873.

Schalm, O.W., Jain, N.C. and Carrol, S.J. (1975). Veterinary haematology, 3rd Edn., Lea and Febriger, Philadelphia.

Sharma, M.C., Umashankar; Gupta, O.P. and Verma, R.P. (1983). Haematological studies in normal cyclic, anoestrus and repeat-breeding crossbred cows. Indian Vet. Med.J., 7:153-155.

Wooton, I.D.P. and Freeman, H. (1982). Microanalysis in medical biochemistry. 6th Edn., Churchill Livingstone, Edinburgh.

IJAR:11:2:120-121:1990

Induction And Synchronization Of Oestrus In True Anoestrus Red Kandhari Cows Treated With Syncro-Mate B Treatment

S. A. JOSHI, D. R. PARGAONKAR, S. A. BAKSHI, S. V. PURANIK and N. M. MARKANDEYA

Department of Gynaecology and Obstetrics, College of Veterinary & Animal Sciences, M.A.U., Parbhani - 431 402

ABSTRACT

Eight dry anoestrus Red Kandhari cows were treated with Syncro-Mate B treatment. The treatment consisted of an ear implant for nine days containing 3 mg norgestomet plus 2 ml SMB intramuscular injection containing 3 mg norgestomet and 5 mg oestradiol valerate on first day. Two ml. injection Prosolvin containing 7.5 mg Luprostiol per ml, a PGF_2 alpha analogue, was also injected with 400 I.U. PMSG injection 48 hrs before removal of ear implant. 100% induction and synchronization of oestrus with subsequent ovulation was recorded in treated cows as against 25% oestrus exhibition in control group. The conception rate on pregnancy diagnosis was recorded to be 25% in treated cows and nil in control group.

• • •

Synchronization of oestrus has been defined as the regulation of oestrus cycle at will. The control and synchronization of oestrus in farm animals has become a subject of major interest in recent years. Controlled breeding can lead to first calving at early age, reduced intercalving period and increased number of calvings resulting in optimum and uniform production. Various methods have been employed for induction and Synchronization of oestrus which include physical, sensorial and protective methods by adequate feeding and management, chemicals and hormones respectively.

Synchro-Mate B is one of the products which constitutes norgestomet ear implant, injection of norgestomet and oestrodiol valerate supported with prostaglandin F_2 alpha and PMSG, hence called as 'Syncro-Mate B plus' treatment.

Materials And Methods

Sixteen Red Kandhari dry anoestrus cows from College Dairy Cattle Unit, Parbhani, were selected to study the efficacy of Syncro-Mate B® treatment. The cows were divided into two groups of 8 each. The treatment was undertaken on group I, whereas Group II served as control. Both the groups were maintained under identical feeding and

IJAR:11:2:122-124:1990

Studies On Involution of Uterus In Relation To Post-Partum Oestrous In Sahiwal X Jersey Cross-Breds With Three Levels Of Inheritance.

A. W. DESHMUKH[1], and A.S. KAIKINI[2].

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

ABSTRACT

The post-partum reproductive perfo-rmance in relation to the uterine involution and appearance of first post-partum oestrous in 70 Sahiwal X Jersey Cross-bred Cows with three levels - 50% (42), 62.5% (14) and 75% (14) of exotic inheritance was investigated. Involution of uterus was significantly faster in primipara than in pluripara in all the cross-bred groups studied. Levels of inheritance also influenced the rate of involution of uterus. The genetic groups, parity groups and farms did not influence the appearance of first post-partum oestrous period. The co-relation between involution of uterus and first post-partum oestrus in Sahiwal X Jersey cross-breds with three levels of inheritance was found to be non-significant.

Materials And Methods

Seventy freshly calved Sahiwal X Jersey Crossbred Cows, with three levels of inheritance 50%, 62.5% and 75.0% maintained at the cattle breeding farms of Veterinary College and Agriculture College, Nagpur were studied.

Gynaeco-Clinical Examination of all the cases, seven days after parturition was undertaken on alternate day during extra-pelvic state of genitalia and daily during intra-pelvic state. Cows having abnormal calvings were excluded from these studies. Involution of uterus was studied as per criteria adopted by Buch *et.al.* (1955) and Kaikini (1983). The parity groups were classified thus : Group-I : Primiparous females; Group-II : Second to fourth calvers and Group-III : Five and above calvers.

The onset on first observed oestrous after calving was detected by external signs. The observations were statistically analysed by applying least square analysis of variance design to study the effect of parity and level of inheritance on the involution of uterus and parity, farm and level of inheritance on the first post-partum oestrous interval.

Results And Discussion

The results indicated that the period for involution of uterus was lowest (28.76 ± 0.75 days) in group-I and highest (35.67 ± 1.14 days) in Group-III animals (Table 1).

The difference between the three parity groups was found to be highly significant ($P<0.01$). Moller (1970) and Kadu and Kaikini (1979) reported similar influence of number of calvings on uterine involution. The half-breds had the lowest average period of involution of uterus (30.61 ± 0.52 days), while animals with 62.5% inheritance showed longer period (34.14 ± 0.68 days). It was highest in animals with 75% inheritance (35.14 ± 1.21 days). The mean values

1. Assistant Professor of Gynaecology.
2. Emeritus Scientist, (ICAR).

(30.61 ± 0.52 days) for involution of uterus in S x J half-breds are lower than those reported by Jana and Mishra (1978) in HF x Sahiwal half-breds (33.40 days) and Bhaskaran *et.al.* (1983) in J x Sindhi half-breds (36.55 days). The difference might be due to genetic variations.

The analysis of variance to test mean differences in first post-partum oestrous interval among the three genetic groups, three parity groups and two farms, with all possible interactions, indicated mean differences among all three sources to be non-significant. The finding of non-significant effect of level of inheritance on first post-partum oestrous is in agreement with Pandey *et.al.* (1979) in J x Hariana crosses and Mahadevan *et.al.* (1984) who reported that, post-partum oestrous interval did not vary significantly among different genetic grades (Jersey/HF/Brown-Swiss Crosses). The present finding of non-significant effect of parity on first post-partum oestrous is in agreement with Yadava *et.al.* (1976) who reported that lactation did not have any effect on the occurrence of first post-partum oestrous, in Jersey Cows. The lowest first post-partum oestrous interval was found in half-breds (115.20 ± 8.59 days), follwed by in 75 per cent inheritance level (121.92 ± 10.96 days) and 62.5 per cent inheritance level (137.23 ± 14.77 days) of Sahiwal x Jersey Cross-bred Cows (Table 1).

The present studies indicated that, co-relation between involution of uterus and first post-partum oestrous period was non-significant. However, these findings differ from those reported by Kadu and Kaikini (1979) who found significant corelation between these two parameters in Sahiwal Cows.

Table 1 : Parity Group and Inheritance Level wise averages of time required for involution of uterus and first post-partum oestrous interval in Sahiwal X Jersey cross-bred females (in days).

Parity Group	Half-bred Jersey X Sahiwal			62.5 percent Jersey X Sahiwal			75 percent Jersey X Sahiwal			Pooled over (Levels)	
	No.of Cows	Involution of uterus (in days)	Ist PP oestrous interval (in days)	No.of Cows	Involu.of uterus (in days)	Ist PP oestrous interval (in days)	No.of Cows	Involu.of uterus (in days)	Ist PP oestrous (in days)	Invo. of uterus (in days)	Ist PP oestrous (in days)
I	10	27.60 ±0.56	124.00 ±16.77	5	32.00 ±0.63	168.25 ±15.70	2	31.50 ±0.50	130.00 ±11.00	28.76 ±0.75	135.12 ±12.12
II	21	30.23 ±0.46	121.33 ±13.77	8	35.00 ±0.68	120.25 ±21.05	9	34.33 ±0.57	114.88 ±12.09	32.21 ±0.65	119.57 ±9.06
III	11	34.09 ±0.73	94.72 ±12.19	1	38.00	149.00	3	40.00 ±4.50	137.66 ±40.38	35.67 ±1.14	107.33 ±24.37
Overall	42	30.61 ±0.52	115.20 ±8.59	14	34.14 ±0.68	137.23 ±14.77	14	35.14 ±1.21	121.92 ±10.96		

REFERENCES

Bhaskran, K.V.; Iyer, C.P.N. and Mahadevan, E. (1983) : Studies on the post-partum reproductive performance of cross-bred Cows. Kerala J. Vet.Sci. **14**:38-43.

Buch, N.C.; Tyler, W.J. and Casida, L.E. (1955) : Post-patrum oestrous and involution of uterus in an experimental herd of Holstein Friesian Cows. J.Dairy Sci. **38**:73-79.

Jana. D. and Mishra, R.R. (1978) : Studies on uterine involution and post-partum oestrous in dairy Cows. Indian J. Dairy Sci. **31**:145-149.

Kadu, M.S. and Kaikini, A.S. (1979) : Involution of uterus in Sahiwal Cows. Mahavet **5**(2):133-135.

Kaikini, A.S. (1983) : Personal communication.

Mahadevan, E.; Naik, K.P. Ravindranathan, N. and Devassia, P.A. (1984) : Certain reproductive traits of cross-bred cows. Indian J.Anim. Reprod. **4**(2):71-74.

Moller, K. (1970) : Uterine involution and ovarian activity during and after calving. N.Z. Vet. J. **18**:140-145. Anim. Breed. Abstr.**39**:397.

Pandey, H.N.; Kaul, G.L. and Katpatal, B.G. (1979) : Post-partum oestrous and weight at calving in cross-bred cows. Indian J.Dairy Sci. **32**(1):11-15.

Yadava, R.K., Conard, H.R. and Gilmore, L.O. (1976) : Effect of body condition at calving on reproductive performance of dairy cow. Indian J.Dairy Sci. **29**:139-141.

IJAR:11:2:124-127:1990

Comparative Efficacy Of Different Serodiagnostic Methods For Detecting Immunologic Infertility In Repeat Breeding Cows

S. BASU [1], S. K. BANDOPADHYAY[2] and R. ROY CHOUDHURY[3]

Department of Animal Gynaecology and Obstetrics, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia-741 252 West Bengal

ABSTRACT

Gelatin agglutination test (GAT), Capillary tube agglutination test (CAT), Slide agglutination test (SAT), Tube slide agglutination test (TSAT), Capillary mucus penetration test (CMPT) and Sperm cervical mucus contact test (SCMCT) were tried to detect immunologic infertility in 32 repeat breeding cows and 2 control cows with normal reproductive cycles. Results showed that GAT, CAT and TSAT except SAT were efficacious for detecting immunologic infertility in repeat breeding cows. CMPT and SCMCT tests showed "Poor" or "Inadequate" sperm penetration and marked "Shaking" phenomenon respectively, suggesting the presence of sperm agglutinin in cervical secretion of repeat breeding cows. It is worth noting that while the serum of repeat breeding animals which were positive reactors, the corresponding mucus samples of these animals became positive also in CMPT and SCMC tests.

• • •

1. House Surgeon, Deptt. of Veterinary Clinics,
2. Reader and Head
3. Professor

Repeat breeding in cows is a major menace affecting livestock economy. Though not always to assess the exact etiology of repeat breeding, the immunologic interference contributes, to a great extent, to this condition. In the present studies an attempt is made to assess the comparative efficacy of different serodiagnostic methods to detect such immunologic interference in repeat beeder cows.

Materials And Methods

A total of 32 repeat breeding cows having five or six regular cycles with apparently normal genitalia and served by A.I. for more than 3 times without conception constituted experimental animals, while 2 cows with normal genitalia and pregnant with one to three A.I. served as control.

Blood samples were collected from all 32 experimental and control cows and serum separated. Cervical mucus samples were also collected from these animals as per the technique of Bhatt *et.al.* (1979). The serum and cervical mucus samples were numbered and stored at 20°C until different serological tests were performed. Positive control rabbit serum (PCRS) having antibodies corresponding to antigen of whole semen was developed and collected from rabbit as per the method recommended by Sundarsanan *et.al.* (1986). This hyperimmune serum served as a positive control for all agglutination tests performed and was run side by side along with the serum samples.

GAT, CAT, SAT, CMPT and SCMCT were performed, respectively, as per the method followed by Kibricks *et.al.* (1952), Franklin and Dukes (1964), Kremer (1965) and subsequently modified by Fallbrant (1968) and Kremer and Jager (1976).

Results And Discussions

On studying the results of different serological tests (Table 1), it is evident that 7 serum samples (21.87%) and the positive control rabbit serum (PCRS) showed positive agglutination in GAT and CAT characterised by the appearance of microscopic white floccules followed by clearing of the suspended medium. On the contrary all the 32 test serum samples (100%) including two controls showed marked head to head agglutination in SAT, thereby suggesting that it should not be used for detecting immunologic causes of repeat breeding. These results corroborate with the findings of Chang (1947); Edwards (1960) and Senger and Saacke (1973) according to whom the serum induced head to head agglutination depended sadly upon the integrity of acrosome and cell membrance of spermatozoa. TSAT was found to be more sensitive than either GAT or CAT because the said test gave a positive agglutination of total 8 test serum samples (25%) which included the 7 test serum samples found positive in GAT and CAT and in addition another test serum sample No. 18. In TSAT, the positive reaction was evidenced by the formation of head to head agglutination as seen under microscope. The intensity or size of the spermatozoal clump formation had a positive correlation with the concentration of anti-spermatozoal antibodies in serum and duration of incubation. The above different agglutination tests clearly indicated that GAT, CAT and TSAT were found reliable for detecting the presence of anti-spermatozoal antibodies in the serum of repeat breeding cows and the positive reactors could be identified as immunologic infertile.

Serological tests with cervical mucus revealed a "poor" or "inadequate" (<15 mm)

sperm penetration in CMPT and more than 80% shaking percentage of SCMC test in 10 cervical mucus samples of repeat breeding cows by decreasing the number of potential spermatozoa at the site of fertilization. It is observed that the cervical mucus of these serum positive reactors became positive in CMPT and SCMC tests. Moreover, sample No. 8 and 11 also gave positive results in these two tests, though they were non-reactive even in TSAT. This might be due to the fact that the sperm agglutinins in these two animals were secreted locally into the cervical mucus directly, instead of through the blood circulation.

Statistical analysis to find out the efficacy of different serological tests performed (Table 2) indicated an overall highly significant χ^2 value of 63.405 (d.f. - 5). However, on analysing the χ^2 values of the different test pairs (d.f. - 1), it was evident that the highly significant variation between the test pairs was due to 100% positive result in SAT (T_3). There was no significant variation in χ^2 values with other test pairs, where SAT was not involved. So, keeping SAT aside, all other tests were equally efficacious, though with CMPT and SCMC test, the positive results were on the higher side.

The immune response evoked in these positive reactors against whole semen was either humoral, cellular (Robers *et.al.* 1978) and local secretory or combination of two or more of these reactions. The infertility in these positive reactors was due to interaction between the antigens of spermatozoa with the long glyco-protein micelles in cervical mucus, in the presence of sperm agglutinin (Kremer and Jager, 1976).

Acknowledgements

The authors are thankful to Dr. P.B. Sarkar, Reader, Deptt. of Microbiology and Dr. S. Biswas, Lecturer, Department of Animal Products and Technology for help and advice and also to Dr. R. Dutta Gupta, Reader, Department of Animal Breeding, BCKV for statistical analysis of results.

REFERENCES

Bhatt, G.N., Vyas, K.K., Purohit, S.K. and Jatkar, P.R. (1979) : Studies of immunofertility in repeat breeder cows. Indian Vet. J. **56**(3):184-188.

Chang M.C. (1947). The effect of serum on spermatozoa. J. Gen. Physiol. **30**(3):321-335.

Edwards, R.G. (1960). Complement fixing activity of normal rabbit serum with rabbit spermatozoa and seminal plasma: J. Reprod. Fert. **10**:268-282.

Franklin R. and Dukes, C.D. (1964) : Antispermatozoal antibody and unexplained infertility. Amer. J. Obst. Gynae. **89**(1):6-10.

Fallbrant, B. (1968) : Interrelation between high levels of sperm antibodies, reduced penetration of cervical mucus by spermatozoa and sterility in men. Acta. Obstet. Gynae. Second. 47-102. Cited by Noel R. Rose (1976).

Kibricks, S., Belding, D.L. and Merill, B. (1952) : Methods for detection of antibiotics against mammalian spermatozoa. II. A gelatin agglutination test. Fertil. and Steril. 3(4):430-438.

Kremer, J. (1965) : A simple Sperm penetration test. Int. J. Fertil. **10**:209.

Kremer, J. and Jager, S. (1976) : The sperm mucus contact test : A preliminary report. Fertil and Steril. **27**(3):335-340.

Noel R. Rose (1976) : Techniques for detection of Iso-and auto-antibodies to human spermatozoa. Clin. Exp. Immunol. **23**:175-179.

Roberts, T.K. and Lewings, E.G. (1978) : Immunological recognition of spermatozoa by female immune system following natural mating. Proc. 4th Int. Symp. on Immunology of Reproduction, Vana, Bulgaria, 19-22 Sept., 1978.

Rumke, P. and Hellinga, G. (1959) : Auto-antibodies against spermatozoa in sterile men. Amer. J. Clin. Path. 32(4):357. Cited by Noel R. Rose (1976).

Senger, P.L. and Saacke, R.G. (1973) : Effects of blood serum on spermatozoa. J. Anim. Sci. 37(1):328. Cited by Noel R. Rose (1976).

Shulman, S. and Hekman, A. (1971). Antibodies to spermatozoa. 1. A new microscopic agglutination technique for their detection using immotile sperm. Clin. Expt. Immunol. 9:137-140.

Sudarsanan, V., Pattabiraman, S.R. and Venkitasami, V. (1986) : Buffalo seminal entibodies : Its detection and titration by gelatin agglutination and tube slide agglutination test. Indian J. Anim. Reprod. 7(2):9-13.

Table 1 : Details of different serological tests

Treatment No.	Name of Serological tests	Positive serum/ mucus sample No.	No.of positive serum/mucus sample	Percentage of positive samples.
T_1	Gelatin agglutination test (GAT)	1,2,6,9,15,24 and 30	7	21.87
T_2	Capillary Tube agglutination (CAT)	1,2,6,9,15,24 and 30	7	21.87
T_3	Slide agglutination test (SAT)	All 32 serum samples	32	100
T_4	Tube slide agglutination test (TSAT)	1,2,6,9,15,18, 24 and 30	8	25
T_5	Capillary mucus penetration test (CMPT)	1,2,6,8,9,11,15, 18,24 and 30	10	31.25
T_6	Sperm cervical mucus contact test (SCMC)	1,2,6,8,9,11,15, 18,24 and 30	10	31.25

Table 2 : X^2 values to test the efficacy of different serological tests.

	T_2	T_3	T_4	T_5	T_6	D.F.
T_1	0	41.03**	0.09	0.72	0.72	1
T_2		41.03	0.09	0.72	0.72	1
T_3			38.4**	33.52**	33.52**	1
T_4				0.31	0.31	1
T_5					0	

Overall - 63.405** ** P<0.01

IJAR:11:2:128-130:1990

Leptospirosis In Repeat Breeding And Anoestrus Cattle

K. KULASEKAR, S. A. QUAYAM and S. RATNAM

Department of Obstetrics and Gynaecology, Madras Veterinary College, Madras-600 007.

ABSTRACT

Sixty one serum samples from cows and buffaloes with problems of repeat breeding and anoestrum were screened for leptospiral infection by microscopic agglutination test using antigens belonging to six serogroups : Autumnalis, Canicola, Grippotyphosa, Hebdomadis, Icterohaemorrhagiae and Pomona. Twenty six out of 61 sera screened revealed antibody titres ranging from 1:20 to 1:320. Three repeat breeding and two anoestrus cattle exhibited a titre of 1:80 and above.

• • •

Leptospirosis is endemic in cattle population of many countries throughout the world (Ellis, 1984). It causes economic loss to the cattle industry from abortion and infertility. In U.K., agglutination test carried out in the last few years on sera from cattle with history of infertility, revelaed the presence of leptospiral antibodies (Michina, 1967). Michina (1970) also observed a chronic form of leptospirosis leading to abortion and breeding difficulties. Early embryonic mortality in cattle could be caused by hardjo infection (Ellis, 1983). In India, occurrence of repeat breeding due to leptospirosis was reported by Rao and Kesavamurthy (1982) and Uppal and Singh (1982).

Inspite of reported prevalence of this disease, no serious attempts have been made to study the role of leptospiral infection in infertility. Many aspects of Leptospirosis in cattle are still inadequately defined. Hence, this investigation was undertaken to determine the incidence of this disease in repeat breeding and anoestrus cattle.

Materials And Methods

Serum samples from 45 cows and 16 buffaloes were collected from repeat breeding and anoestrus cases that attended Madras Veterinary College Hospital.

Cows and buffaloes with apparently normal genital organs but did not conceive to three or more successive services or A.I. were considered as repeat breeders.

Cows and buffaloes with soft and smooth ovaries which did not show oestrum for 120 days after parturition were considered as anoestrus.

Seven to ten day old cultures of leptospires grown in E_mJH medium were inactivated by addition of formaldehyde solution to a final concentration of 0.2 per cent v/v (Turner, 1968). These inactivated cultures were used as antigen for the test. Titre value of 1:80 and above was considered positive for leptospiral infection (Ratnam *et.al.* 1980).

Serum samples collected from the above group of animals were screened by microscopic agglutination test (Wolff, 1954). The six leptospiral killed antigens used were : (i) Autumnalis, (ii) Canicola (iii) Grippotyphosa, (iv) Hebdomadis, (v) Icterohaemorrhagiae and (vi) Pomona.

Results And Discussion

26 out of 61 serum samples showed titres ranging from 1:20 to 1:320. In repeat breeding, 7 samples exhibited a titre of 1:20, 6 a titre of 1:40 and one each against 1:80, 1:60 and 1:320. In anoestrum, 7 showed titre of 1:20 and one each showed a titre of 1:40, 1:80 and 1:320. The serogroups encountered in order were Autumnalis, Hebdomadis, Icterohaemorrhagiae, Pomona, Canicola and Grippotyphosa.

Michina (1970) in his review on leptospirosis reported that serogroups Pomona, Icterohaemorrhagiae, Grippotyphosa and Canicola may be responsible for breeding difficulties in cattle. The prevalence of antibodies to serogroups varies from region to region that is Hardjo in Australia (Elder and Ward, 1978; Autumnalis, Grippotyphosa and Pomona in Tamil Nadu (Ratnam *et.al.* 1980) and Pomona, Shermani, Canicola and Hebdomadis in Karnataka State (Rao and Kesavamurthy, 1982).

In the present study Autumnalis predominated, which may be due to its common prevalence as indicated by Ratnam *et.al.* (1983, 1987) who isolated leptospira belonging to Autumnalis serogroup in man and bandicoots from Madras City. Repeat breeding cows exhibited antibodies largely at dilutions of 1:20 to 1:40 and less at higher dilutions of 1:80 and above. Ellis *et.al.* (1985) reported that sero var hardjo can persist in non-pregnant bovine oviduct and uteri for at least 91 and 83 days post-infection respectively. Similar persistence of other sero var in genital tract of non-pregnant bovine may be expected. This is further supported by the observation of Ellis *et.al.* (1986) showing a lower serological prevalence that microbiological prevalence in the genital tract of cattle suggesting that these infections were of long standing duration. Repeat breeding condition being a type of chronic localised infection affecting the genital tract may probably be the reason for low antibody titres observed in this study.

In Anoestrum, 3 cows and 7 buffaloes showed titres ranging from 1:20 to 1:320. The incidence of antibody reaction was comparatively less in cows. Ellis *et.al.* (1986) reported the presence of carrier state of leptospira in the oviduct, uteri and ovaries of naturally infected non-pregnant cattle. The association of leptospires with anoestrum has not been proven. The persistance of leptospira in the oviduct, uterus, and ovaries as indicated by Ellis *et.al.* (1986) provides a possible theoretical basis for anoestrum which may require further investigation.

REFERENCES

Ellis, W.A. (1983). Recent developments in bovine Leptospirosis. The Vet. Annu. 23rd issue Scientechnica.

Ellis, W.A. (1984). Bovine Leptospirosis in tropics: Prevalence, Pathogenesis and Control. Prev. Vet. Med. **2**:411-421.

Ellis, W.A., O'Brien, J.J., Cassells, J.A., Neill, S.D., and Hanna, J. (1985). Excretion of Leptospira interrogans servovar hardjo following calving or abortion, Res. Vet. Sci. **39**:296-298

Ellis, W.A., Songer, J.G., Montgomery, J. and Cassells, J.A. (1986). Prevalence of Leptospira interrogans servovar hardjo in the genital and urinary tracts of non pregnant cattle. Vet. Rec. **118**:11-13.

Elder, J.K., and Ward, W.H. (1978). The prevalence and distribution of leptospiral titres in cattle and pigs in Queensland. Aust. Vet. J. **54**:297-300.

Michna, S.W. (1967). Animal leptospirosis in the British Isles: A serological survey. Vet. Rec. **80**:394.

Michna, S.W. (1970). Leptospirosis. Vet. Rec. **86**:484 496.

Rao, A.S., and Kesavamurthy, B.S. (1982). Serological evidence of leptospiral infection in buffaloes. Indian J. Comp.

Microbiol Immunol and Infec. Dis. 3:147-152.
Ratnam, S., Sundararaj, T., and Subramanian, S. (1980). Serological evidence of Leptospirosis among cattle. Proc. Fourth National Congress of Med. Microbiol. Vol. IV. 104-107.
Ratnam, S., Subramanian, S., Madanagopalan, N., Sundarraraj, T., and Jayanthi, V. (1983). Isolation of Leptospires and demonstration of antibodies in human leptospirosis in Madras, India. Trans. Royal. Soc. of Trop. Med. and Hyg. 77(4):455-458.
Ratnam, S., Sundararaj T., Subramanian, S., and Adinarayanan, N. (1987). Experimental study with Leptospires in Bandicoots. Indian J. of Expt. Biol. 25:105-107.
Turner, L.M. (1968). Leptospirosis II, Serology. Trans. Roy. Soc. of Trop. Med and Hyg. 62:880.
Uppal, P.K., and Singh, S.P., (1982). Note on the sero prevalence of leptospirosis in buffaloes Indian. J. of Anim. Sci. 52:1247-1249.
Wolff, J.W. (1954). The laboratory diagnosis of Leptospirosis. Pub. Charles C. Thomas, Springfield, Illinois.

IJAR:11:2:130-135:1990

Effect Of Levels Of Trace Elements In Estrual Cervical Mucus On Fertility In Mehsani Buffaloes.

V. P. VADODARIA[1] and G. A. PRABHU[2]

Gujarat Agricultural University, Sardar Krushinagar-385 506.

ABSTRACT

Trace elements levels in estrual cervical mucus (CM) were quantitated in relation to fertility, physiological status, and physical characteristics of CM in Mehsani buffaloes.

Zn^{++}, Mn^{++}, Fe^{++} levels differed significantly ($P<0.01$) and remained higher in conceived group while the levels of Cu^{++} remained significantly ($P< 0.01$) higher in non-conceived group.

CM of buffaloes as compared to heifers, had significantly ($P<0.01$) lower level of Cu^{++} when the samples were studied at different stages of estrus (early, mid and late) and at different degrees of tone of uterus (++, +++ scores). Trace elements content fluctuated non-significantly with other characteristics; lactational status; colour and consistency of CM. Higher levels of Cu^{++} by virtue of its spermicidal effect might have reduced chances of conception in non-conceived group. On other hand higher levels of Zn^{++}, Mn^{++} might have favoured the chances of conception in Mehsani buffaloes and heifers. The status of individual trace elements or the definite relation between different trace elements along with other substances might be deciding factors in regulating fertility.

• • •

Trace elements act as co-factors and activators or as stabilizers of secondary molecular structures (Valee and Wacker, 1976). Studies on human seminal plasma revealed that depending upon the concentrations, the divalent cations (Ca^{++}, Mg^{++} and

1. Professor of Physiology.
2. Professor of Physiology and Biochemistry, College of Veterinary Science and A.H., Anand-388 001 Gujarat.

Zn^{++}) could exert stimulatory or inhibitory effect on sperm activity (Stegmayr and Ronquist, 1982). Further it has been reported that excessive amount of copper present in media surrounding sperm (seminal plasma) could act as a toxic metal to human sperm (Umeyama *et al.* 1986). Gupta *et al.* (1985) reported non-significant difference in the concentration of Zinc and Iron in cervico-vaginal mucus of fertile and repeat breeder cows. However reports are lacking on levels of various trace elements in CM of buffaloes and their relationship with fertility. The present investigation therefore, was carried out on composition of trace elements in relation to physical (colour, consistency) and physiological (lactational status, estrus intensity, estrus stage and tone of uterus) conditions in order to assess their significance by using them as diagnostic indices in female infertility/subfertility in Mehsani buffaloes.

Materials And Methods

Healthy cyclic Mehsani buffaloes and heifers brought for A.I. were selected for present study. Prior to collection of CM, information comprising age, lactational status, number of lactation, time and intensity of estrus were recorded. CM samples were collected as per Reddy, (1973) and uterine tone recorded.

Animals were examined 3 months post-insemination and divided into conceived group and non-conceived group. The state and number of lactation was based on the history provided by the buffalo owner. Intensity of estrus was classified as +, ++ and +++ scores considering the estrus expression. The estrus phases were divided into early, mid and late estrus. The tone of uterus was recorded as + (mild), ++ (moderate) and +++ (Good) scores, depending on P.R. examination and the degree of myometrial contraction resulting in rigidity, tonicity, turgidity and coiling of uterus.

The colour of CM was categorized as clear, turbid and opaque (Deo and Roy, 1971). Its consistency was classified as thin and thick (Reddy, 1973). Techtron Atomic Absorption Spectrophotometer, Model-AA 120 was used for estimating Zinc (Zn^{++}), Iron (Fe^{++}), Copper (Cu^{++}) and Magnaese (Mn^{++}) from acid digested aliquote of CM. 5 ml of diluted CM was mixed with 15 ml of acid digestion mixture consisting of 1 volume perchloric acid (60 to 62%) to 4 volumes of nitric acid (69 to 71%) and CM sample was digested as per the acid digestion procedure for biological materials (Krishna and Ranjhan, 1981).

Standard statistical procedures were applied for analysis of the data, using a factorial experiment under CRD and t-test for testing of overall pooled mean values (Snedecor and Cochran, 1980). Data of conceived and non-conceived groups were analysed separately for lactational status, intensity and stage of estrus, colour and consistency of mucus and tone of uterus. Buffaloes and heifers were considered as different 'classes'.

Results And Discussion

The mean values, representing six characteristics of conceived and non-conceived groups when pooled and tested, revealed

significantly higher levels of Zn^{++} ($P<0.01$), Fe^{++} ($P<0.01$), and Mn^{++} ($P<0.01$) but lower level of Cu^{++} ($P<0.05$), in CM of conceived group as compared to non-conceived group. The mean values in CM of conceived group were 3.58 ± 0.10, 0.28 ± 0.10, 2.41 ± 0.10, and 1.71 ± 0.07, mg% for Zn^{++}, Mn^{++}, Fe^{++} and Cu^{++}, respectively. The corresponding values in CM of non-conceived group were 2.27 ± 0.10, 0.12 ± 0.004, 1.60 ± 0.0.06 and 2.10 ± 0.14, mg%, respectively (Tables 1,2). It seems from available literature that there are no reports on trace elements values of estrual CM of buffaloes. On the basis of present findings, it could be presumed that higher levels of Zn^{++}, Fe^{++} and Mn+ in CM had favourable effect on fertility in Mehsani buffaloes. Further, higher level of Cu^{++} in CM of non-conceived group of animals in the present study in suggestive of possible spermicidal effect of Cu^{++}. Umeyama *et.al.* (1986) reported toxicity to spermatozoa due to higher level of Cu^{++} in seminal plasma. Mann (1964) described the powerful spermicidal effect of Cu^{++} by sulphydryl binding.

Overall Mn^{++} levels in CM were lower compared to other trace elements studied. Even, sizeable number of samples did not contain detectable amount of Mn^{++} in CM of Mehsani buffaloes and heifers.

The ANOVA of mean values (Table 1) show that none of the physical and physiological characters studied separately during present investigation could affect significantly the level of any of the trace elements. Significant treatment effect shown for Cu^{++} when partitioned for various main effects (groups, classes and levels) were not found to be significant. It is possible that there might be some inter-actions which could not be revealed since these were not included as a component of statistical analysis.

Cervical mucus of buffaloes on an average was found to contain lower values of Cu^{++} in comparision to CM of heifers ($P<0.01$). Overall Cu^{++} value of CM of buffaloes and heifers was 1.62 ± 0.01 and 2.65 ± 0.06 mg%, respectively. This variation was suggestive of process of poor stabilization of cervix/genital tract of heifers since they have not experienced pregnancy changes.

Although the trace elements levels increased non-significantly with increase in estrus intensity scores, mid-estrual mucus had higher Zn^{++}, Mn^{++} and Fe^{++} in conceived group as compared to non-conceived group. Compared to early and late stages of estrus, highest levels of aforesaid three trace elements were recorded during mid-estrus. However, Cu^{++} levels in CM revealed opposite trends when compared between groups and estrus stages. The mean Cu^{++} concentrations in CM of conceived group were 2.07 ± 0.69, 1.81 ± 0.49 and 1.61 ± 0.35 mg% for early, mid and late heat, respectively. The corresponding values were 1.63 ± 0.84, 2.04 ± 0.65 and 6.96 ± 0.00 mg% respectively.

Acknowledgement

Authors are thankful to Dr. V.M. Jhala, Principal and Dean, College of Veterinary Science and A.H., Sardar Krushinagar for providing necessary facilities.

Table 1 : Average ($\bar{X}$ ± S.E.) Zinc and Manganese concentration (mg%) in cervical mucus of conceived and non-conceived animals under different physical and physiological characters.

Characteristics	Zn** (mg%)		Mn** (mg%)	
	Conceived group	Non-conceived group	Conceived group	Non-conceived group
1. Lactational status				
(a) Lactating	4.85 ± 0.92 (20)	2.30 ± 0.62 (20)	0.19 ± 0.02 (15)	0.12 ± 0.02 (18)
(b) Non-lactating	3.38 ± 0.53 (29)	2.34 ± 0.75 (13)	0.19 ± 0.02 (17)	0.13 ± 0.03 (4)
2. Intensity of estrus				
++	1.80 ± 0.63 (7)	1.67 ± 0.66 (11)	0.16 ± 0.10 (5)	0.09 ± 0.02 (5)
+++	3.75 ± 0.57 (35)	2.71 ± 0.67 (22)	0.20 ± 0.10 (25)	0.14 ± 0.02 (16)
3. Stage of estrus				
Early heat	3.67 ± 0.61 (11)	1.61 ± 0.20 (7)	0.15 ± 0.10 (6)	0.05 ± 0.00 (1)
Mid heat	3.58 ± 0.61 (33)	2.60 ± 0.66 (23)	0.90 ± 0.69 (25)	0.11 ± 0.02 (17)
Late heat	1.87 ± 1.44 (2)	0.25 ± 0.00 (1)	0.18 ± 0.07 (2)	----
4. Colour of mucus				
Clear	4.58 ± 0.79 (9)	1.59 ± 0.75 (10)	0.22 ± 0.17 (10)	0.16 ± 0.01 (3)
Turbid	2.89 ± 1.40 (21)	2.74 ± 0.80 (11)	0.18 ± 0.10 (14)	0.13 ± 0.02 (8)
Opaque	2.79 ± 0.61 (13)	2.28 ± 0.64 (12)	0.22 ± 0.14 (8)	0.13 ± 0.92 (6)
5. Consistency of mucus				
Thin	3.60 ± 0.60 (33)	1.92 ± 0.63 (23)	0.21 ± 0.10 (22)	0.11 ± 0.01 (13)
Thick	3.05 ± 0.66 (12)	3.21 ± 0.84 (8)	0.16 ± 0.10 (10)	0.17 ± 0.04 (6)
6. Tone of uterus				
++	2.47 ± 0.66 (10)	1.26 ± 0.47 (11)	0.19 ± 0.14 (9)	0.07 ± 0.04 (4)
+++	3.83 ± 0.58 (35)	2.72 ± 0.73 (18)	0.19 ± 0.10 (22)	0.12 ± 0.02 (11)
Overall	3.58 ± 0.10	2.27 ± 0.10**	0.28 ± 0.10	0.12 ± 0.004**

Note : Figures in parentheses indicate number of animals.

** P< 0.01

Table 2 : Average ($\bar{X}$ ± S.E.) Iron and Copper concentrations (mg%) in cervical mucus of conceived and non-conceived animals under different physical and physiological characters.

Characteristics	Fe** (mg%)		Cu** (mg%)	
	Conceived group	Non-conceived group	Conceived group	Non-conceived group
1. Lactational status				
(a) Lactating	2.46 ± 0.53 (14)	1.81 ± 0.52 (20)	1.50 ± 0.30 (21)	1.43 ± 0.51 (21)
(b) Non-lactating	2.16 ± 0.65 (21)	0.43 ± 0.07 (9)	2.16 ± 0.45 (26)	3.33 ± 1.02 (9)
2. Intensity of estrus				
++	1.85 ± 0.41 (6)	1.56 ± 0.73 (9)	1.29 ± 0.61 (13)	1.75 ± 0.94 (8)
+++	2.53 ± 0.71 (27)	1.82 ± 0.70 (17)	1.49 ± 0.47 (36)	2.68 ± 0.58 (24)
3. Stage of estrus				
Early heat	2.56 ± 0.95 (6)	0.76 ± 0.56 (5)	2.07 ± 0.69 (12)	1.63 ± 0.84 (4)
Mid heat	2.39 ± 0.60 (27)	1.65 ± 0.62 (21)	1.81 ± 0.49 (35)	2.04 ± 0.65 (23)
Late heat	0.50 ± 0.00 (1)	0.50 ± 0.00 (1)	1.61 ± 0.35 (3)	6.96 ± 0.00 (1)
4. Colour of mucus				
Clear	3.52 ± 0.90 (9)	1.17 ± 0.75 (8)	1.12 ± 0.20 (11)	1.07 ± 0.41 (8)
Turbid	2.30 ± 0.73 (14)	2.31 ± 0.74 (12)	1.14 ± 0.39 (19)	2.89 ± 0.98 (9)
Opaque	2.71 ± 0.91 (8)	1.13 ± 0.57 (8)	3.23 ± 0.85 (9)	0.43 ± 0.16 (8)
5. Consistency of mucus				
Thin	2.12 ± 0.61 (22)	1.61 ± 0.61 (22)	1.73 ± 0.50 (36)	2.35 ± 0.62 (21)
Thick	3.05 ± 0.67 (11)	1.89 ± 0.69 (5)	1.93 ± 0.54 (13)	1.67 ± 0.79 (8)
6. Tone of uterus				
++	2.75 ± 0.72 (10)	1.38 ± 0.65 (10)	1.82 ± 0.46 (13)	1.61 ± 0.66 (7)
+++	2.03 ± 0.59 (23)	1.89 ± 0.69 (17)	1.63 ± 0.48 (35)	2.55 ± 0.69 (20)
Overall	2.41 ± 0.10	1.60 ± 0.06**	1.71 ± 0.07	2.10 ± 0.14 *

Note : Figures in parentheses indicate number of animals.

* $P < 0.05$

** $P < 0.01$

REFERENCES

Deo, S. and Roy, D.J. (1971). Investigation on repeat breeding cows and buffaloes - Studies on physical properties of cervical mucus. Indian Vet. J. 48(5):479-484.

Gupta, R.C. Luthra, R.A. Baru, P. and Krishnaswamy, A. (1985). Concentration of iron zinc in the cervico-vaginal mucus of fertile and repeat breeder cows. Indian J. Anim. Reprod. 6(2):26-28.

Krishna, G. and Ranjhan, S.K. (1981). Laboratory manual for nutritional research. Vikas Publication House Pvt. Ltd.

Mann, T. (1964). The biochemistry of semen and of the male reproductive tract. Methuen and Co. Ltd., London, John Wiley and Sons. Inc. New York.

Reddy, V.N. (1973). Studies on certain physical and biochemical properties of cervical mucus of bovines in estrus. M.V.Sc. Thesis, University of Agricultural Sciences, Bangalore.

Snedecor, G.W. and Cochran, W.C. (1980). Statistical methods. 7th Edn., The Iowa State University Press, Ames Iowa, U.S.A.

Stegmayr, B. and Ronquist, G. (1982). Stimulation of Sperm motility by Organelles in human seminal plasma. Scand J. Urol. Nephrol. 16:85.

Umeyama, T., Ishikawa, H., Takeshima, H., Yoshii, S., Koiso, K. (1986). A comparative study of seminal trace elements in fertile and infertile men. Fertil. Steril. 46(3):494-499.

Valee, B.L. and Wacker, W.E.C. (1976). In The proteins 4.0 Neurath, ed. Vol. 5 : Academic Press : New York.

IJAR:11:2:135-140:1990

Purification Of Inhibitor(s) From Buffalo Follicular Fluid To Compensatory Ovarian Hypertrophy In Mice

S. KUMAR[1] and H. C. PANT[2]

Department of Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry Mathura-281002

ABSTRACT

Subcutaneous administration of 0.3 and 0.6 ml whole and charcoal treated buffalo follicular fluid inhibited compensatory ovarian hypertrophy in ovariectomized mice. The follicular fluid was salted-out with 18.5% (W/V) ammonium sulphate and the salted-out fraction was separated into two fractions by Sephadex G-200 column chromatography. Both fractions detectable as a single band by polyacrylamide gel disc electrophoresis, possessed inhibitory activity. However, second fraction showed greater inhibitory activity which was destroyed following trypsin digestion.

• • •

Inhibin, a heterodimetric protein of gonadal origin which preferentially suppresses FSH synthesis and secretion (Mc Lachlan *et.al.* 1988) has been isolated from pig (Ling *et.al.* 1985; Miyamato *et.al.* 1985) and cow follicular fluid (Forage *et.al.* 1986; Robertson *et.al.* 1985, 1986). Among the domestic animals, inhibin-like activity has been

1. Scientist S-1, Livestock Production Research, I.V.R.I., Izatnagar-243122 (U.P.)
2. Address reprint requests to Professor H.C. Pant

demonstrated in the follicular fluid of ewe (Tsonis *et.al.* 1983; Findlay *et.al.* 1985), cow (de Jong and Sharpe, 1976) and mare (Channing *et.al.* 1981). Steroid free extracts of bovine and porcine follicular fluid have been shown to reduce plasma FSH concentrations in the rat (de Jong and Sharpe, 1976; Hopkinson *et.al.* 1977; Marder *et.al.* 1977; Welschen *et.al.* 1977). A purified fraction from bovine and porcine follicular fluid has been demonstrated to inhibit compensatory ovarian hypertrophy in the ovariectomized mice (Sato *et.al.* 1978; 1982). The object of the present experiment was to test whether a similar activity was also present in the buffalo follicular fluid.

Materials And Methods

i) *Buffalo follicular fluid* : Ovaries were obtained from adult buffaloes within 1 hr of their slaughter at a local abattoir during late winter and transported to the laboratory in chilled (4°C) physiological saline (0.154 M Nacl). Follicular fluid was aspirated from all visible antral follicles within 4 hr of slaughter, using a tuberculin syringe fitted with a 24 gauze needle. A pooled sample of follicular fluid was then centrifuged at 6000 r.p.m. for 20 minutes at room temperature (about 18°C). The supernatant (whole follicular fluid) was separated and stored at -4°C until required. Before use, a portion of the whole follicular fluid was stirred with charcoal (50 mg/ml) at room temperature for 20 minutes and then left in the refrigerator overnight. The charcoal was then separated next morning by centrifugation and filtration (Whatman No. 41 paper) of the supernatant.

ii) *Purification of the inhibitor* : A fraction of the whole follicular fluid was treated with 18.5% (W/V) ammonium sulphate. It was kept for 4 hr at 4°C, then centrifuged at 6,000 r.p.m. for 40 minutes at room temperature. The precipitate was suspended in distilled water and dialysed against distilled water for 24 hr at 4°C. Contents of dialysis tubing were centrifuged and the supernatant was freeze dried. The freeze dried material was dissolved in 1 ml distilled water and a 0.5 ml aliquot was loaded on to a column of Sephadex G-200 (1.5 x 100 cm, bed height 85 cm). Elution was carried out with distilled water at room temperature at a flow rate of about 6.5 ml/hr and fractions of 2 ml were collected in separate tubes. Protein content in each fraction was measured as per Lowry *et.al.* (1951) and fractions constituting a peak were pooled and freeze dried. Polyacrylamide gel disc electrophoresis was performed as per Davis (1964).

iii) *Examination of compensatory ovarian hypertrophy (COH) in mice* : Young adult IVRI strain mice were kept at room temperature under natural day-light schedule with free access to feed and water. They were unilaterally ovariectomized under ether anaesthesia between 1100 and 1200 hr on the day of dioestrus. The left ovaries were removed and weighed on a torsion balance to the nearest 0.1 mg. The whole follicular fluid, charcoal treated follicular fluid, fractioned samples from follicular fluid and trypsin treated fraction (50 µg trypsin/mg protein) were injected subcutaneously into the experimental mice, while the control mice received 0.154 M-NaCl. Each mouse received either 0.3 or 0.6 ml of follicular fluid or 200 µg of protein in an aliquot of 0.6 ml. Each group consisted of 5 mice and each experiment was replicated once. 72 hours after the operation the mice were killed and the right ovaries were removed and weighed.

The degree of compensatory ovarian hypertrophy (COH) was calculated as a percentage increase in the weight of the right ovary (at sacrifice) compared with that of the left (at hemispaying).

Results

Hypertrophy of the right ovary was significantly suppressed in the mice injected with 0.3 or 0.6 ml of the whole follicular fluid or with 0.6 ml of the charcoal treated follicular fluid. The degree of suppression was significantly greater with 0.6 than with 0.3 ml whole follicular fluid and was similar to that observed with equivalent volume of charcoal treated follicular fluid (Table 1).

The precipitate salted out with 18.5% ammonium sulphate was separated into two peaks by gel chromatography (Fig 1). The freeze-dried material of both peaks significantly suppressed the COH in the mice (Table 2). However, while there was no significant difference between 100 and 200 µg doses of peak 1 (fractions 21 to 35), injection of 200 µg doses the material from peak 2 (fractions 50 to 62) significantly suppressed the ovarian hypertrophy in comparison to 100 µg dose of the same peak. Trypsin digestion of the material from peak 2 abolished this suppressive activity.

Patterns of polyacrylamide gel disc electrophoresis of the whole follicular fluid and the concentrated samples from peak 1 and 2 are shown in Plate 1. Samples from both peaks appeared as a single band. However, the band of peak 1 was behind the albumin fraction of the whole follicular fluid while that of peak 2 was ahead of the albumin fraction.

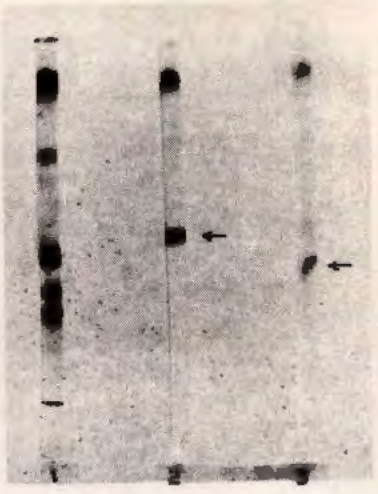


Plate 1 : Patterns of polyacrylamide gel disc electrophoresis at pH 8.3 of (1) whole buffalo follicular fluid, (2) Peak 1 and (3) Peak 2 (Fractions 21-35 and 50-62, see Fig. 1) arrowed.

Discussion

Injection of 0.3 and 0.6 ml of the whole follicular fluid suppressed the COH in the mice, suggesting that buffalo follicular fluid contains inhibitory substance(s) as reported earlier in the procine (Sato *et.al.* 1978) and bovine (Sato *et.al.*, 1982) follicular fluid. This inhibitory activity is unlikely to be due to the oestrogen and progesterone content of the follicular fluid. Charcoal treatment of the follicular fluid is known to remove 95 to 99% of these steroids (Schwartz and Channing, 1977). In our study the charcoal treated follicular fluid was as effective as the whole follicular fluid in suppressing COH in the mice. It is, therefore, suggested that this suppressive effect of the whole follicular fluid may be due to factor(s) other than steroids. This suggestion receives support from the observation that the activity was retained in the materials obtained after purification. Furthermore, loss of activity of

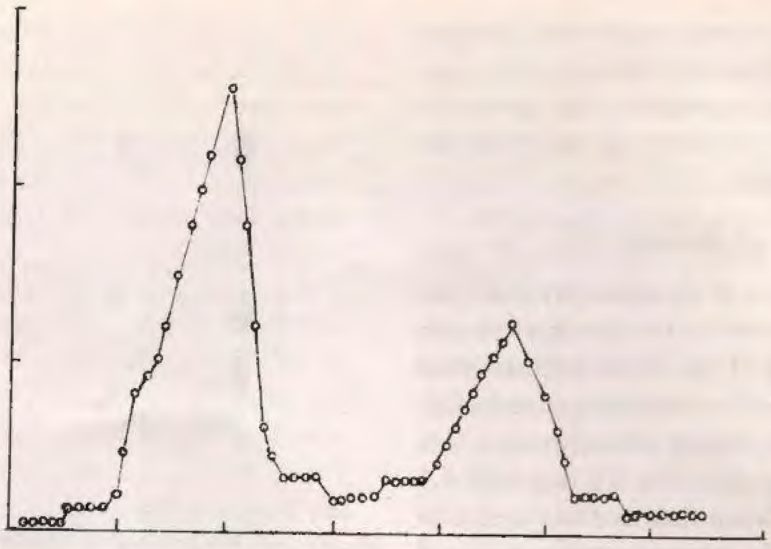


Figure 1. Gel filtration of the fraction of buffalo follicular fluid salted out at 18.5% ammonium sulphate on Sephadex G-200 column chromatography.

peak 2 following trypsin digestion confirms its proteinaceous nature.

In an earlier study Japanese workers also obtained two protein peaks following Sephadex G-200 gel filtration of ammonium sulphate salted-out cow follicular fluid (Sato *et.al.*, 1982). However, these investigators found COH suppressing activity only in the material from peak 2 while we found the inhibitory activity in both peaks. This may be due to the presence of two separate inhibitors in the buffalo follicular fluid.

Several investigators have observed that an inhibin-like substance in the bovine (de Jong and Sharpe, 1976, Hopkinson *et.al.*, 1977) and porcine (Marder *et.al.*, 1977; Welschen *et.al.*, 1977) follicular fluid has the ability to decrease serum FSH values. Additionally, follicular fluid inhibits the binding of FSH to its receptors on the granulosa cells (Darga and Reichert, 1978; Sato *et.al.*, 1982). It is, therefore, tempting to speculate that of the two protein fractions present in the buffalo follicular fluid, one may suppress COH by decreasing circulating FSH levels and the other by suppressing FSH activity at the ovary. Futher studies are warranted to test this suggestion.

Acknowledgement

We thank the Dean, College of Veterinary Science and Animal Husbandry, Mathura for providing facilities.

REFERENCES

Channing, C.P., Batta, S.K., Kenney, R.M., Ganjam, V.K. and Condon, W. (1981). Levels of atretogenic factor and inhibin-F in atretic and viable equine follicular fluid. In Dynamics of Ovarian Function, pp 73-78. Eds. N.B. Schwartz and M. Hunzicker-Dunn. Raven Press, New York.

Darga, N.C. and Reichert, L.E. (1978). Some properties of the interaction of follicle stimulating hormone with bovine granulosa cells and its inhibition by follicular fluid. Biol. Reprod. **19**:235-241.

Davis, B.J. (1964). Disc electrophoresis II. Method and application to human serum proteins. Ann. New York Acad. Sci. **121**:404-410.

De Jong, F.H. and Sharpe, R.M. (1976). Evidence for inhibin-like activity in bovine follicular fluid. Nature, Lond.

263:71-72.

Findlay, J.K., Gill, T.W. and Doughton, B.W. (1985). Influence of season and sex on the inhibitory effect of ovine follicular fluid on plasma gonadotrophins in gonadectomized sheep. J. Reprod. Fert., **73**:329-335.

Forage, R.G., Ring, J.M., Brown, R.W., Mc Inerney, B.V., Cobon, G.S., Gregson, R.P., Robertson, D.M., Morgan, R.J., Hearn, M.T.W., Findlay, J.K., Wettenhall, R.E.H., Burger, H.G. and de Kretser, D.M. (1986). Cloning and sequence analysis of cDNA species coding for the two subunits of inhibin from bovine follicular fluid. Proc. Natn. Acad. Sci., U.S.A. **83**:3091-3095.

Hopkinson, C.R.N., Daume, E., Sturm, G., Fritze, E., Kaiser, S. and Hirschhauser, C., (1977). Inhibin-like activity of bovine ovarian extracts in male and female rats. J. Reprod. Fert. **50**:93-96.

Ling, N., Ying, S.Y., Ueno, N., Esch, F., Denoroy, L. and Guillemin, R. (1985). Isolation and partial characterization of a Mr 32000 protein with inhibin activity from porcine follicular fluid. Proc. natn. Acad. Sci., U.S.A. **82**:7217-7221.

Lowry, O.H., Rosebrough, N.H., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the folin phenol reagent. J. biol. Chem. **193**:265-275.

Marder, M.L., Channing, C.P. and Schwartz, N.B. (1977). Suppression of serum follicle stimulating hormone in intact and acutely ovariectomized rats by porcine follicular fluid. Endocrinology, **101**:1639-1642.

McLachlan, R.I., Robertson, D.M., de Kretser, D.M. and Burger, H.G. (1988). Advances in the physiology of inhibin and inhibin-related peptides. Clin. Endocr. **29**:77-144.

Miyamoto, K., Hasegawa, Y., Fukuda, M., Nomura, M., Igarashi, M., Kangawa, K. and Matsuo, M. (1985). Isolation of porcine, follicular fluid inhibin of 32 k Daltons. Biochem. Biophys. Res. Commun. **129**:396-403.

Robertson, D.M., Foulds, L.M. Leversha, L., Morgan, F.J., Hearn, M.T.W., Burger, H.G., Wettenhall, R.E.H. and de Kretser, D.M. (1985). Isolation of inhibin from bovine follicular fluid. Biochem. Biophys. Res. Commun., **126**:220-226.

Robertson, D.M., de Vos, F.L., Foulds, L.M., Burger, H.G., Morgan, F.J., Hearn, M.T.W. and de Kretser, D.M. (1986). Isolation of a 31 kDa form of inhibin from bovine follicular fluid. Molec. Cell. Endocr., 44:271-277.

Sato, E., Ishibashi, T. and Iritani, A. (1982). Purification and action sites of a follicle stimulating hormone inhibitor from bovine follicular fluid. J. Anim. Sci., **55**:873-877.

Sato, E., Miyamoto, H., Ishibashi, T. and Iritani, A. (1978). Identification, purification and immano-histochemical detection of the inhibitor from porcin ovarian follicular fluid to compensatory ovarian hypertrophy in mice. J. Reprod. Fertil. **54**:263-267.

Scwartz, N.B., and Channing, C.P. (1977). Evidence for ovarian inhibin: suppression of the secondary rise in serum follicle stimulating hormone level in proestrous rats by injection of porcine follicular fluid. Proc. Natn. Acad. Sci., USA **74**:5721-5724.

Tsonis, C.G., Quigg, H., Lee, V.W.K. Leversha, L., Trounson, A.O. and Findlay, J.K. (1983). Inhibin in individual ovine follicles in relation to diameter and atresia. J. Reprod. Fert., **67**:83-90.

Welschen, R., Hermans, W.P., Dullaart, J. and De Jong, F.H. (1977). Effects of an inhibin-like factor present in bovine and porcine follicular fluid on gonadotrophin levels in ovariectomized rats. J. Reprod.Fert., **50**:129-131.

Table 1 : Effects of whole and charcoal treated buffalo follicular fluid on compensatory ovarian hypertrophy in mice (Mean ± S.E.M.)

Treatment	Dose (ml/mouse)	No. of mice treated	Weight of left ovary (mg)	% hypertrophy of right ovary
Saline (Control)	0.6	10	2.93 ± 0.26	80.1 ± 4.8
Whole follicular Fluid	0.3	10	2.89 ± 0.22	49.9 ± 5.5*
	0.6	10	3.12 ± 0.33	16.3 ± 4.4**
Charcoal treated follicular fluid	0.6	10	3.40 ± 0.17	16.8 ± 4.3**

* Significantly different from the control value ($P<0.01$, Student's t-test)

** Significantly different from the control value ($P<0.01$), and from the 0.3 ml whole follicular fluid value ($P<0.01$, Student's t-test).

Table 2 : Effects of the fractions obtained by gel filtration on compensatory ovarian hypertrophy in mice (Mean ± S.E.M.)

Treatment	Dose (µg/mouse)	No. of mice treated	Weight of left ovary (mg)	% hypertrophy of right ovary
Saline (Control)	0.6 ml	10	2.93 ± 0.26	80.1 ± 4.8
	100	10	3.6 ± 0.28	46.3 ± 7.7*
Peak 1	200	10	3.7 ± 0.20	35.6 ± 9.3*
	100	10	4.0 ± 0.36	39.5 ± 9.8*
Peak 2	200	10	3.3 ± 0.20	15.9 ± 4.3**
Trypsin digested Peak 2	200	10	3.7 ± 0.24	61.9 ± 8.6

* Significantly different from the control value (P<0.01, Student's t-test)

** Significantly different from the control value (P<0.01), and from the 100 µg equivalent fraction value (P<0.05, Student's t-test).

IJAR:11:2:140-141:1990

Variations In Serum Progesterone Profiles In Fertile And Repeat Breeder Buffaloes (Bubalus bubalis) During Late Oestrus

N.S. BUGALIA[1] and R.D. SHARMA

Department of Obstetrics and Gynaecology, College of Veterinary Sciences,
Punjab Agricultural University, Ludhiana - 141004.

ABSTRACT

Blood samples were collected from 20 fertile and 20 repeat breeder buffaloes during late oestrus and serum progesterone profiles were monitored by radio-immuno--assay (RIA). Non-significant variations were recorded in serum progesterone levels between fertile (0.49 ± 0.07 ng/ml) and repeat breeder (0.35 ± 0.05 ng/ml) buffaloes.

* * *

Repeat breeding without clinical signs of genital disease is a common expression of infertility in bovines (Ayalon, 1973). Sequential biochemical changes in the female genital tract provide receptive milieu for spermatozoa and ovum during ovulation period under the influence of oestrogen and progesterone (Broth *et.al.* 1957). Cyclic variations in plasma progesterone profiles have been reported in buffaloes (Bachlaus *et.al.* 1979; Takkar *et.al.* 1983). Inadequate literature on significance of progesterone in buffalo fertility warranted to undertake present investigation.

Materials And Methods

A total of 20 fertile and 20 repeat breeder buffaloes were sampled for blood serum

1. Assistant Professor, Department of Gynaecology and Obstetrics, College of Veterinary Sciences, Haryana Agricultural University, Hisar-125004.

during late oestrus for RIA of progesterone and subsequently inseminated with frozen semen. Both fertile and repeat breeder buffaloes had regular oestrous cycle without genital pathology but fertile buffaloes conceived following single insemination during late oestrus, whereas repeat breeder buffaloes failed to conceive with past three inseminations including the sampling oestrus. Serum progesterone was measured by RIA technique as per Thorneycroft and Stone (1972). The coefficient of intra and inter assay variation was 10.21 and 14.63% respectively. The sensitivity of assay was 9 pg per tube. Statistical analysis of data was done by standard methods (Snedecor and Cochran, 1967).

- **Results And Discussion**

The observed non-significant variations in serum progesterone profiles between fertile (0.49 ± 0.07 ng/ml) and repeat breeder (0.35 ± 0.05 ng/ml) buffaloes confirmed observations of Arora *et.al.* (1979) in pregnant (0.17 ng/ml) and non-pregnent (0.13 ng/ml) buffalo heifers during oestrus. In fertile buffaloes, Ahmed *et. al.* (1977) also reported 0.50 ng per ml serum progesterone level during oestrus. However, Dugwekar *et.al.* (1975) and Ahmed *et.al.* (1977) found higher (1.0 ng/ml) and lower (0.10 ng/ml) serum progesterone level in repeat breeder buffaloes during oestrus, respectively.

Present study indicates insignificance of progesterone profiles during late oestrus in predicting the fertility status in buffaloes. Non-significant variations in progesterone levels were also reported in fertile and repeat breeder cows from oestrus to till day 4 (Ayalon, 1973) and till day 8 (Erb *et.al.* 1976) post-insemination. Cervical and endometrial milieu is influenced by oestrogen during oestrus (Broth *et.al.* 1957). Thus variations in fertility among fertile and repeat breeder buffaloes could be ascribed to differences in oestrogen profiles during late oestrus altering cervical and endometrial medium affecting transport of spermatozoa and implantation of blastocyst.

Acknowledgements

Authors are grateful to Dr. S.S. Gill, Head, Animal Science Department, Punjab Agricultural University, Ludhiana for providing buffaloes. Senior Research Fellowship awarded to the first author by I.C.A.R., New Delhi is gratefully acknowledged.

REFERENCES

Ahmed, A. Agarwal, S.P., Agarwal, V.K., Rahman, S.A. and Laumas, K.R. (1977). Steroid hormones : Part II-Serum progesterone concentration in buffaloes. Ind. J.Exp. Biol. **15**:1591-1593.

Ayalon, N. (1973). Comparative studies of repeat breeders and normal cows. In: Annual Report Res. No. 2 pp 1-5. Kimron Vet. Institute, Beit Dagan (Israel).

Bachlaus, N.K., Arora, R.C., Prasad, A. and Pandey, R.S. (1979). Plasma level of gonadal hormones in cycling buffalo heifers. Ind.J.Exp.Biol. **17**:823-825.

Broth, R., Lunenfelf, B. and De Watteville, H. (1957). Day-to-day variation in urinary gonadotropin and steroid levels during the normal menstrual cycle. Fertil. Steril. **8**:233-39.

Dugwekar, Y.G., Rao, B.R., Bapat, S.T. and Varman, P.N. (1975). Estrogen and progesterone levels in a repeat breeder buffalo. In : Proc. Symp. "Use of radiations and radio-isotopes in studies of Animal Production". pp. 270-275. Indian Vet. Res. Institute, Izatnagar-243122.

Erb, R.E., Gaverick, H.A., Rendel, R.D., Brown, B.L. and Collahan, C.J. (1976). Profile of reproductive hormones associated with fertile and non-fertile inseminations of dairy cows. Theriogenol. **5**:227-242.

Snedecor, G.W. and Cochran, W.G. (1967). Statistical methods. 6th ed. pp. 59, 228, 258, Oxford and I.B.H., Calcutta (India).

Takkar, O.P. Singh, M. and Varman, P.N. (1983). Seasonal variations in serum progesterone levels in cycling and non-cycling buffaloes. J. Nucl. Agri. Biol. **12**:7-10.

Thorneycroft, I.H. and Stone, S.C. (1972). Radioimmuno-assay of serum progesterone in women receiving oral contraceptive steroids. Contracept. **5**:129-146.

IJAR:11:2:142-144:1990

White Side Test For Subclinical Metritis In Buffaloes

A. K. PATERIA[1] and C. V. S. RAWAL[2]

Animal Reproduction Division, Indian Veterinary Research Institute, Izatnagar-243122 (U.P.)

ABSTRACT

White side test was carried out on uterine discharge of 100 buffaloes to detect subclinical cases of metritis. The Test reactions were divided into 3 categories on the basis of intensityof colour developed viz. Slight, Moderate and Intensive positive. All the clinical cases of metritis gave intense positive reaction, subclinical cases gave all three types of reactions and normal buffaloes did not show any reaction. The pH of the uterine discharge was also found to be higher in clinical and subclinical metritis cases than in normal buffaloes.

* * *

Metritis in buffaloes is quite prevalent throughout the world causing infertility (Kodagali *et.al.* 1980) and resulting into great economic loss. In the available literature there is not much information on white side test as a diagnostic test for subclinical metritis in buffaloes. Popov (1969) used white side test on cervical mucus of cows for diagnosis of occult endometritis. Cervical mucus was heated with Sodium hydroxide solution upto the boiling point. The reaction was considered positive if the colour turned yellow. There was a corelation between the number of leucocytes present in the mucus and the intensity of the yellow colour. In the present study white side test was used to differentiate the subclinical cases of metritis from normal buffaloes.

Materials And Methods

One hundred visiting buffaloes to Key Village Centre I.V.R.I., for A.I. were taken randomly to study the incidence of subclinical and clinical metritis. White side test was standardized first and then it was used to detect subclinical cases of metritis. A series of sodium hydroxide solution (range 1 to 10%) were prepared and one ml each was combined with 1 ml of uterine discharge of clinical metritis case, upto boiling point. Five percent Sodium hydroxide solution gave the best results. The appearance of yellow colour was taken as a positive indication of metritis. The colour reaction was also positive where the cases were not showing apparent symptoms of metritis (Subclinical Metritis). Those samples which were negative for colour reactions were considered as normal. The uterine discharges were further categorised as slight, moderate and intense subclinical metritis discharge on the basis of colour viz. Light yellow, Yellow and Dark yellow, respectively. pH of all mucous samples was determined by use of paper strips (BDH).

Results And Discussion

The test was used to differentiate the normal healthy animals from subclinical and

1. M.V.Sc. Student.
2. Senior Scientist (SG).

clinical cases of metritis. The results are presented in Table 1. The status of metritis was highly correlated with the different intensities of test reactions. The test reactions were divided into 3 categories.

S Slight positive - Light yellow colour
M Moderate positive - Yellow colour
I Intense positive - Dark yellow colour.

The uterine discharge from subclinical cases were positive for all the three types of colour reactions i.e. slight, moderate and intense, whereas the normal uterine discharge did not show any reaction and the clinical metritis cases showed only intense reactions.

All the clinical cases of metritis fell in the category I and the difference was significant ($P<0.05$) from S and M categories. However, no significant difference ($P>0.05$) was observed in category S and M. The cases in subclinical group were significantly ($P<0.05$) higher in category M than S and I. However, the cases were equal in the S and I categories but they differ significantly ($P<0.05$), whereas normal uterine discharge did not show any reaction. This could be explained on the basis of number of leucocytes present in the uterine discharge (Popov, 1969). The normal discharge has too low number of leucocytes to cause any change of colour whereas subclinical discharges have moderate number of leucocytes causing a moderate colour change compared to the clinical discharge having very high number causing intense colour reaction.

A slight increase in the pH values of uterine discharge was recorded in buffaloes showing subclinical and clinical metritis respectively (Table 2). This is in agreement with the findings of Boitor *et.al.* (1980) for cows.

Table 1 : Distribution of clinical and subclinical metritis cases.

Type	Severity			X^2 Value
	S	M	I	
Clinical	0	0	100	28.12*
Subclinical	25	50.00	25.00	

* Significant at 1% probability level ($P<0.01$).

Table 2 : Test of significance for pH.

Parameter	Type of Animal		
	Clinical	Subclinical	Normal
pH of uterine discharge	8.80 ± 0.06^a (15)	8.23 ± 0.06^b (60)	7.26 ± 0.82^c (25)

Note : 1. Means with common superscripts are not significantly different at 5% probability level ($P>0.05$).
2. Figs. in parenthesis show number of animals.

REFERENCES

Boitor, I., Muntean, M., Mutes, N., Moise, D., Morar, R., Boitor, M., Sahleanu, C., and Gorgan, L. (1980). Laboratory and therapeutic Studies of pureperal endometritis and repeat breeding in the cow. Buletinul Institutuls Argonomic Clúg-N apoea Zoo-technic Si Medicine Veterinara, 34:111-114 C.F. Vet. Bull (1982), 52:5715.

Kodagali, S.B., Bhavsar, B.K., Kavani, F.S., Derasari, H.J., Mansuri, A.N., Dholakia, P.M. Purohit, J.H. and Shah, N.M. (1980). Clinical trials with 'Furea Bolus' in endometritis and repeat breeder buffaloes, Indian Vet. J. 57(11):945-950.

Popov, Yu. N. (1969). Diagnosis of occult endometritis in cows (using white side test on cervical mucus) Veterinariya Moscow No. 4 pp 85-87.

IJAR:11:2:144-145:1990

Effect of Iodine Therapy In Anoestrous Bovines

K.D. RYOT, B.K. SHARMA, and C.D. PANWAR

Hill Cattle Development Centre, Kandaghat-173215 Himachal Pradesh

ABSTRACT

Findings of Iodine therapy in anoestrous hill cattle and buffaloes are reported. Over-all oestrus response of 70% in treated cases indicates iodine administration as an effective remedy for anoestrus condition in bovines of hill areas.

* * *

The cyclic activity in the female is a complex process mainly governed by hypothalamic- pituitary- ovarian axis. Iodine deficiency can depress thyroid function affecting fertility indirectly, and hypo or hyperthyroidism may reduce the secretion of gonadotrophic hormones by the anterior pituitary (Sane *et.al.* 1982). Iodine deficiency has been attributed to anoestrous in cows and buffaloes in Tarai area of Uttar Pradesh (Dabas *et.al.* 1987). Himachal Pradesh, a hilly state, is largely prone to the deficiency of iodine in soil. The higher incidence of anoestrus in both hilly and cross-bred bovines in this zone incited this study.

Materials And Methods

172 anoestrous animals including post parturient cows and buffaloes and heifers of breedable age were used in this study. The gynaeco-clinical examination of these animals revealed the absence of any pathological condition of reproductive tract and the non-functional quiescent ovaries. These animals were divided into 3 groups. Group I animals (33 cows and 32 buffaloes) were drenched 10ml of Lugol's iodine each diluted with water, daily for 10 days. Group II animals (42 cows and 33 buffaloes) in addition had 5 ml of 0.25% iodine solution sprayed on the cervix on first day of the treatment. Group III animals (18 cows and 14 buffaloes) were not given any treatment and were treated as control.

The animals were kept under observation for 30 days from inception of treatment for appearance of oestrous signs and those found in oestrus were inseminated. Pregnancy was confirmed by rectal palpation after 60 days of insemination.

Results And Discussion

Ovulatory oestruses, mostly with overt signs, were observed in responding animals between 9 to 30 days of start of the treatment. 72.72% cows and 62.50% buffaloes came in oestrus in Group I, whereas, 73.80% and 69.69% cows and buffaloes respectively were observed in oestrus in Group II. In Group III the oestrus responses in cows and buffaloes were 5.55% and 7.14% respectively. Out of the responding animals, 66.66% cows and 55.00% buffaloes conceived in Group I, and 64.51% cows and 60.86% buffaloes conceived in Group II.

The oestrous responses in both the treated groups were significantly higher (P±0.05) than the control group and appreciably good conception responses in both the groups strengthen the observations of Moberg (1961). The overall oestrous response (70.00%) in the present study was lower than that (73.13%) reported by Dabas *et.al.* (1987), which may be due to comparatively shorter course of treatment in this study. The higher percentages of oestrus and conception in Group II animals as compared to Group I might be due to the local action of iodine as remarked by Singh *et.al.* (1987) in goats.

REFERENCES

Dabas, Y.P.S., Varshney, A.C. and Saxena, O.P. (1987). Influence of iodine supplementation on reproductive performance in bovines of Tarai area. Indian Vet. J. **64**(11):950-952.

Moberg, R. (1961). Possible influence of supplementary iodine administered by evaporation on reproductive performances in cattle. In Veterinary Obstetrics and Genital Diseases by S.J., Robers, 2nd Edn. CBS Publishers and Distributors, Delhi. p. 457.

Sane, C.R., Deshpande, B.R. and Marathe, M.R. (1982). Infertility in cows and buffaloes. In Reporduction in Farm Animals by C.R. Sane, S.N. Luktuke, B.R. Deshpande, A.S. Kaikini, D.P. Velhankar, V.B. Hukeri and S.B. Kodagali, 1982 Edn. Varghese Publishing House, Bombay. p. 292.

Singh, D.K., Sinha, M.P. and Singh, R. (1987). Studies on efficacy of Secrodyl, Prajana and Lugol's paint on anoestrous goats. Indian Vet. J. **64**:(2)178-179.

IJAR:11:2:145- :1990.

Biometrics Of Gravid Genitalia In Bannur Ewes

S.R. CHINCHKAR, D.P. VELHANKAR, B.R. DESHPANDE and V.L. DEOPURKAR

Department of Animal Reproduction, Bombay Veterinary College, Parel, Bombay-400012.

ABSTRACT

Observations on gravid genitalia of 18 Bannur ewes were recorded in 1st, 2nd, 3rd and 4th month of gestation period in respect of circumstances of uterine cornua, ovaries, placentomes, amniotic and allantoic fluids. The observations include both the gravid as well as non-gravid cornua. Biometrical studies of these parameters are discussed.

• • •

The studies on prenatal development of farm animals are useful to evaluate normal variations and to precisely understand the specific effects of environmental factors in the development of embryos or foetuses in utero.

The present observations were carried out on clinically normal gravid uteri from 18 Bannur ewes, irrespective of their age and parity. The ligaments and extraneous tissues were dissected out and the weights of the gravid uteri recorded. The embroys or foetuses along with their amnion, chorion and allantois were separated. The quantity of amniotic and allantoic fluids were measured with the help of measuring cylinder. The biometrical observations on dissected gravid genitalia were recorded as per De-Lange (1950). The data was subjeeted to standard statistical treament as per Snedecor and Cochran (1967).

Results And Discussion

1. *Side of gravidity* : Out of 18 Bannur ewes studied, left side pregnancy was encountered in 10 organs (55.56%), whereas 8 organs showed right side pregnancy (44.44%). This indicates that pregnancy occurs fairly equal in both horns in ewes.

2. *Circumference* : The circumference of uterine cornua on the gravid side steadily increases from 8.16 ± 0.17, 20.92 ± 1.46, 26.92 ± 0.30 cm and 38.33 ± 0.73 from 1st to 4th month of gestation period. The non-gravid horn also showed concurrent increase from 7.0 ± 0.0, 14.5 ± 0.18, 18.17 ± 0.28 and 18.83 ± 0.16 cm during 1st to 4th month of gestation period (Table 1). The changes in gravid genitalia are due to enormous increase in blood and lymph vessels with increase in size and number also (Cloete, 1939). During second half of pregnancy, gravid horn outgrows the other, resulting in enlargement and changes in shape (Curson and Quinlan, 1934; Curson and More, 1934). Similar observations are recorded in the present study.

3. *Ovaries* : The weight of ovary on gravid side was larger 0.66 ± 0.09 to 1.36 ± 0.15 g. The corresponding figures for the ovaries on the non-gravid side were 0.53 ± 0.05 to 0.67 ± 0.09 g (Table 1). The increase in the weight of the ovaries on gravid side may be due to the presence of fuctional corpus luteum. Similar observations were reported by Cloete (1939).

4. *Placentomes* : Sheep have cotyledonary placetomes made up of concave maternal caruncles enclosing the convex foetal caruncle. The total number of placentomes varies 65.33 ± 7.23 to 81.67 ± 6.50 (Table 1). This is in agreement (84.33) with Cloete (1939) and that (88 to 96) reported by Ellenberger and Baum (1921).

The diameters of placentomes increase steadily during 2nd, 3rd and 4th month of gestation. The size of placentomes recorded were 2.10 ± 0.15, 2.80 ± 0.19 and 2.07 ± 0.26 during 2nd, 3rd and 4th month of gestation respectively. Similar observations (0.28 to 3.0 cm.) were recorded by Cloete (1939). The gravid horn invariably contains larger number of placentomes, whereas the number in non-gravid horn depends upon the extension of allantois chorion on that side.

5. *Foetal Fluids*: The amniotic and allantoic fluids showed steady increase from 1st to 4th month of gestation period. The total volume of fluids was 24.83 ± 0.60, 168.08 ± 36.11, 390.50 ± 46.54 and 621.67 ± 46.45 ml during 1st, 2nd, 3rd and 4th month of gestation (Table 1).

These figures are in agreement with Cloete (1939). The bulk of amniotic fluid which increases gradually determines the shape of pregnant uterus, when middle portion of pregnant horn appears dilated whereas the portions near the body and apex are comparatively narrow. Cloete (1939), Malan and Curson (1937) also reported that the total amount of fluid increases steadily with advancing foetal age.

Table 1 : Biometrics Of Gravid Genitalia In Bannur Ewes (in cms.)

Gestation period in months	Statistics	Circumference of uterine cornua		Weight of ovaries in g		Placentomes in 'gravid horn'			Placentomes in non-gravid horn			Pacentomes	Allantoic fluid	Amniotic fluid	Total volume
		Gravid	Non-gravid	Gravid	Non-gravid	Total placentomes	Max. size	Above 2 cm. diameter	Total placentomes	Max. size	Above 2 cm. diameter	Grand Total	Volume ml	Volume ml	of foetal fluids ml
First Month (n=3)	Mean	8.16	7.0	1.125	0.53	45.33	—	—	36.33	—	—	81.67	22.5	2.33	24.83
	S.E.	0.17	0.0	0.10	0.05	3.85	—	—	3.29	—	—	6.50	0.28	0.33	0.60
Second Month (n=6)	Mean	20.92	14.5	1.36	0.67	37.0	2.1	6.17	33.33	2.13	4.33	70.33	135.67	33.42	168.08
	S.E.	1.46	0.18	0.15	0.09	3.52	0.15	1.70	3.35	0.15	1.36	6.34	31.88	6.03	36.11
Third Month (n=6)	Mean	26.92	18.17	1.15	0.60	35.17	2.80	8.67	33.67	2.49	8.16	68.83	180.83	209.67	390.5
	S.E.	0.30	0.28	0.20	0.13	2.19	0.19	2.56	2.04	0.48	3.15	3.48	21.38	46.65	46.54
Fourth Month (n=3)	Mean	38.33	18.83	0.66	0.53	33.33	2.07	11.33	32.0	2.1	8.67	65.33	443.33	178.33	621.67
	S.E.	0.73	0.16	0.09	0.24	1.67	0.26	0.88	2.52	0.21	1.20	4.18	36.71	11.68	46.45

REFERENCES

Cloete, J.H.L. (1939). Prenatal growth in the Merino sheep, Onderstepoort J. Vet. Sci. and Anim. Ind. 13:417-558.

Cole, N.N. and Cupps, P.T. (1959). Reproduction in Domestic Animals, Vol. I, Academic Press, New York and London.

Curson, H.H. and Mare, G.S. (1934). Studies on sex Physiology No. 12. The situation of the developing foetus and the relationship between the pregnant horn and the corresponding corpus luteum verum, Onderstepoort J. Vet. Sci. and Anim. Ind. 3(2):477-486.

Curson, H.H. and Quinlan, J.B. (1934). Studies on sex Physiology No. 10 the situation of the developing foetus in Merino sheep Onderstepoort, J. Vet. Sci. and Anim. Ind. 2(2):657-663.

De lange, M. (1950). The influence of delayed breeding on the fertility of beef heifers. Onderstepoort J. Vet. Sci. and Anim. Ind. 24:125.

Ellenberger, W. and Baum, H. (1921). Cited by Cole and Cupps (1959).

Malan, A.P. and Curson, H.H. (1937). Studies on sex Physiology No. 18, on the growth of the gravid uterus in the Merino sheep, Onderstepoort J. Vet. Sci. and Anim. Ind. 8(2):417-427.

Snedecor, G.W. and Cochran, W.G. (1967). Statistical methods, 6th Edn. The Iowa State University Press, Ames, Iowa, U.S.A.

IJAR:11:2:148-152:1990

Induction Of Parturition In Goats With Prostaglandin F_2 Alpha

M.S. THAKUR and S.K. VERMA[1]

Department of Gynaecology and Obstetrics, College of Veterinary Sciences, Haryana Agricultural University, Hisar-125004.

ABSTRACT

Ten pregnant goats (Beetal and Beetal x Black Bengal crosses) were divided into two groups of 5 each. Parturition was induced in experimental group with Prostaglandin F_2alpha (100 µg), while the other group served as control.

The average interval from injection to parturition in treated group was 162.2 ± 8.47 hrs. and 29.0 ± 1.41 hrs. in the control group. The average gestation length, as a consequence, was low (139.8 ± 0.49 days) in treated group as compared to untreated group (149.0 ± 2.15 days). There were no adverse effects of induction of parturition on placental expulsion, kid weight, kid survival and postpartum fertility in does. Periparturient withdrawl of plasma progesterone and gradual increase in estradiol to a peak level on the day of parturition were observed in both normal and induced cases.

* * *

The induction and synchronization of parturition offers many managerial and clinical advantages in different species of animals. In goats, synchronization of kidding reduces the incidence of kid-death due to lack of assistance at the time of parturition, limit kidding in a defined period, thereby facilitating the supervision of process (Williams, 1982). Prostaglandins or its synthetic analogues have been shown to be luteolytic

1. Professor

in goats by a number of workers and hence can be used to induce parturition.

Materials and Methods

Ten pregnant Beetal and Beetal x Black Bengal cross goats in advanced stages of pregnancy (135-140 days) were selected for the study. They were divided into two groups of five each serving : As control (saline treated) and experimental group in which parturition was induced between 135-140 days of pregnancy with 100 μg of synthetic prostaglandin F_2alpha (R-ILIREN, HOECHST AG. FRANKFURT CM) injected intramuscularly.

The interval from injection to parturition, the average gestation length, time required for expulsion of placenta, kid weight, kid survival upto 15 days and postpartum fertility of does were studied. The periparturient profiles of progesterone and oestradiol 17-B were monitored by RIA during normal and induced parturition.

Blood sampling: In control group, blood was collected from all the animals once every day from five days antepartum to 2 days postpartum. In experimental group blood was collected two days before treatment to 2 days postpartum. Blood collection was done by jugular venipuncture in a sterilized heparinized graduated centrifuge tubes between 7 to 8 AM. The blood samples were centrifuged at 1500 rpm for 15 minutes. The plasma was separated and stored as separate aliquots of 5 ml each at -20°C till assayed.

Hormone Assay : Plasma concentration of progesterone and oestradiol 17-B were monitored using RIA I 125 coat-A-count kits supplied by M/s Diagnostic Products Corporation, U.S.A. The antiserum were highly specific for steroids assayed.

Results And Discussion

The average interval from the commencement of therapy to parturition was significantly less (29.0 ± 1.41 hours) in induced group as compared to control (162.2 ± 8.47 hours) (Table 1). By virtue of its luteolytic effect and consequent fall in progesterone levels PGF_2alpha induce early parturition in goats (Wentzel *et.al.* 1978). Day and Southwell (1979) and Bretzlaff and Ott (1983) have reported similar interval from commencement of therapy to induced parturition. However, Walker (1983) and Jain and Madan (1982) employing 100 μg cloprostenol and 20 mg. dinoprost respectively, observed longer interval than observed in present study, which may possibly be due to differences in breeds and body weight vs. dose of the drug/active ingradient.

Consequent upon early induction of parturition, the average gestation length was significantly low (139.8 ± 0.49) in induced as compared to control (149.0 ± 2.15 days) group.

Placenta was expelled at an average interval of 9.7 ± 0.89 hours and 5.5 ± 0.89 hours in induced and control group respectively (Table 1), without cases of retention of after birth in any group. Jain and Madan (1982), William (1982) and Walker (1983) have also found that there is no problem of retention of placenta with prostaglandin F_2alpha induced parturition. The reasons for higher time taken for expulsion in induced group in pre-

sent study are not clear.

Kid weights were 1.06 ± 0.09 kg. and 2.36 ± 0.33 in induced and control groups respectively. There was no kid mortality in any of the two groups for an observation period of 15 days. Similar opinions have been framed by several workers (Lucas and Notman, 1974; Bretzlaff and Ott 1983; Walker, 1983 and Jain and Madan, 1982).

The progesterone concentration showed a decline from a prepartum value of 6.66 ± 0.23 ng/ml on day 5 antepartum to 0.52 ± 0.15 ng/ml on the day of parturition, which further dropped to 0.08 ± 0.01 ng/ml on day 2 post partum in normal parturition in goat (Table 2). A similar trend of progesterone concentration was observed in induced parturition with PGF_2alpha. The progesterone values dropped from 7.05 ± 0.17 ng/ml on day 3 antepartum to 0.70 ± 0.16 ng/ml on the day of parturition. The values dropped further to 0.09 ± 0.02 ng/ml on day 2 postpartum. As reported earlier by various workers (Van Rensburg, 1971; Rowling and Ward, 1973, 1978), periparturient withdrawal of progesterone was observed in present study. In induced group, an abrupt fall in the progesterone concentration was noted within 24 hours of injection which corroborates with the findings of Thorburn and Currie (1973); Goldberz and Ramwel (1976), Currie (1974, 1975) but differ from the findings of Irving *et.al.* (1972) who reported a gradual decline in plasma concentration of progesterone before delivery in normal group.

The estradiol concentration showed a gradual increase from a pre-partum value of 35.88 ± 8.03 pg/ml on day 5 antepartum to a peak value of 946.07 ± 250.00 pg/ml on the day of parturition, which subsequently dropped to 41.49 ± 12.40 pg/ml on the day 2 postpartum in normal parturition. In induced group the values rose from 54.02 ± 12.92 pg/ml on day 3 antepartum to a peak value of 519 ± 144.94 pg/ml on the day of parturition and dropped to 249.57 ± 73.15 pg/ml on day 2 postpartum (Table 2). The peak level of estradiol in normal parturition was recorded on the day of parturition, the peak in induced group was observed on one day after parturition and postpartum values were much higher in induced as compared to control. Findings of present study are in agreement with those of Jain and Madan (1982) and Challis and Linzel (1971) who also reported a similar trend of gradual increase to peak value in the level of estradiol on the day of parturition and then subsequent decline in the postpartum period. In normal parturition higher level of oestradiol induces luteolysis by stimulating PGF_2alpha production. Present findings are not in agreement with those of Thorburn *et.al.*(1972) and Umo *et.al.*(1976) who reported no change in estradiol concentration.

Acknowledgements

Thanks are due to Dr. R.C. Gupta, Dean, College of Veterinary Sciences, HAU for providing the facilities and constant encouragement. Authors are also thankful to Dr. Daya Singh, then Professor and Head and Dr. A.K. Kanojia, Associate Professor, Department of Animal Breeding, College of Animal Sciences, HAU, Hisar for conducting the experiment.

Table 1 : Induction of parturition in goats withProstaglandin F_2alpha, effect on expulsion of placents, kid weight, kid survival and postpartum fertility of goats.

Group	No.of Animals	Interval from injection to parturition (Hrs.)	Av. gestation length (Days)	Time required for expulsion of placenta (Hrs.)	Kid weight (kg.)	Kid survival 15 days	Post partum fertility
I Control	5	162.2±8.74	149.0±2.15	5.5± 0.89	2.36± 0.33	100%	100%
II Induced	5	29.0±1.41	139.8±0.49	9.7±0.89	1.06±0.09	100%	100%

Table 2 : Plasma profiles of progesterone and oestradiol-17 β in normal and induced parturition. (Mean ± S.E.)

Day	Progesterone		Oestradiol-17 β	
	Group I (Control) ng/ml	Group II (Induced) ng/ml	Group I (Control) pg/ml	Group II (Induced) pg/ml
-5	6.66 ± 0.23	—	35.58 ± 8.03	—
-4	5.42 ± 0.45	—	57.11 ± 12.39	—
-3	5.12 ± 0.74	7.05 ± 0.17	81.91 ± 14.22	54.03 ± 12.92
-2	4.95 ± 0.85	6.63 ± 0.81	114.57 ± 19.73	127.95 ± 41.81
-1	5.57 ± 0.92	5.97 ± 0.82	248.35 ± 66.48	250.29 ± 110.43
0	0.52 ± 0.15	0.70 ± 0.16	946.07 ± 66.48	519.36 ± 144.94
+1	0.20 ± 0.03	0.27 ± 0.04	96.04 ± 19.95	603.18 ± 141.15
+2	0.08 ± 0.01	0.09 ± 0.02	41.49 ± 12.40	249.25 ± 73.15

REFERENCES

Bosu, W.T.K., Garibay, J.A.S. and Barker, C.A.V. (1979). Peripheral plasma levels of progesterone in pregnant goats and in pregnant goats treated with prostaglandin F_2alpha Theriogenology **11**:131-148.

Bretzlaff, K.N. and Ott, R.S. (1983). Doses of prostaglandin F_2alpha effective for induction of parturition in goats. Theriogenology **19**:849-853.

Challis, J.R.G. and Linzell, J.L. (1971). The concentration of total unconjugated estrogens in the plasma of pregnant goats. J. Reprod. Fertil. **26**:401-404.

Currie, W.B. (1974). Foetal and maternal hormone interaction controlling parturition in sheep and goats. Ph.D. thesis, Macquire Univ. Sydney (c.f. Ciba foundation symposium. The fetus and Birth p. 57).

Currie, W.B. (1975). Secretion rate of prostaglandin F during induced labour in goats. Prostaglandin **9**:867-869 (c.f. Ciba foundation symposium). The fetus and Birth, p.51.

Day, A.M. and Southwell, S.R.G. (1979). Termination of pregnancy in goats using cloprostenol. Newzeland Vet. J. **27**:207-208.

Goldberg, V.T. and Ramwel, P.W. (1976). Role of prostanglandin in reproduction. Physiologic review. **55**:325-351.

Irving, G., Jones, D.E. and Knifton, A. (1972). Progesterone concentration in the peripheral plasma of pregnant goats. J. Endocrinology **53**:441-442.

Jain, G.C. and Madan, M.L. (1982). Effect of prostin F_2alpha and dexamethasone on prostaglandin F_2alpha and oestradiol 17 β level during induced parturition in goats. In proceedings of 3rd Inter. Conf. on goat prod. and disease. Jan. 10-15, 1982. Tucson, Arizona, (U.S.A.).

Lucas, J.M.S. and Notman, A. (1974). The use of corticoids to synchronize parturition in sheep. Brit. Vet. J. **130**:1-5.

Rowlings, J.C. and Ward, W.R. (1978). Effect of foetal hypophysectomy on the initiation of parturition in goats.

J. Reprod. Fertil. **52**:249-254.

Rowlings, N.C. and Ward, W.R. (1973). The effect of administration of progesterone on the initiation of parturition in goats. J. Reprod. Fertil. **35**:606-607.

Thorburn, G.D. and Currie, W.B. (1973). The physiology of initiation of labour in sheep (C.F. in the fetus and Birth) Ciba foundation symposium No. 47 Ed. J. Knight and M.C. Connor, p. 66, North Holland.

Umo, J. Fitzpatric, R.J. and Wood, W.R. (1976). Parturition in goats, plasma concentration of prostaglandin F_2alpha and steroid hormones and uterine activity during late pregnancy and parturition J. Endocr. **68**:383-389.

Van Rensberg, S.J. (1971). Reproductive physiology and endocrinology of normal and habitually aborting angora goats. Onderstepoort J. Vet. Res. **38**:1-62 (c.f. the fetus and birth, Ciba foundation symposium No. 47 Ed. J. Knight and M.C. Conner, p. 49: North Holland).

Walker, F.M.M. (1983). Lactation and Fertility in goats after induction of parturition with analogue of prostaglandin F_2alpha-cloprostenel. Res. Vet. Sci. **34**:280-286.

Wentzel, D., Calliers, J.J.E. and Botha, L.J.J. (1978). Time course of decreasing progesterone levels in prostaglandin treated Angora goat doe. Agnoanimalia **10**:55.

Williams, S.F. (1982). Induction of parturition in goats using prostaglandin. Lab. Anim. Sci. **52**:451.

IJAR:11:2:152-155:1990

Serum Progesterone Profiles Around Parturition In Surti And Marwari Goats*

A.V. PATEL[1], M.M. PATHAK[2], V.M. MEHTA[3] and K. JANAKIRAMAN[4]

Reproductive Biology Research Unit, College of Veterinary Science and Animal Husbandry, Gujarat Agricultural University, Anand Campus, Anand-388 110.

ABSTRACT

Employing standard RIA technique, serum progesterone of pregnant Surti and Marwari goats was estimated from 7th day antepartum to 20th day post-partum. The average concentrations of serum progesterone around parturition for Surti and Marwari goats was 3.61 and 3.56 ng/ml respectively. The overall trend of progesterone concentration antepartum and post-partum remained almost similar for both the breeds.

The blood concentration of progesterone at day 7 antepartum for Surti and Marwari goats was 9.30 and 9.18 ng/ml respectively, which gradually declined and stabilised to 3.23 and 2.88 ng/ml respectively, a day before kidding. A precipitous fall in progesterone level was recorded on the day of kidding. In post-partum period, progesterone content slowly decreased and remained almost at a basal level upto 20 days in Surti and Marwari goats.

The variations in progesterone level between different stages around parturition were statistically significant ($P<0.01$) for

* Research project on Goat Reproduction financed by PL-480 USDA, Grant No. FG-IN-643.

1. Assistant Research Scientist. 2. Associate Research Scientist. 3. Research Scientist and Head.
4. Director of Research and Dean, P.G. Studies, Gujarat Agricultural University, Ahmedabad.

both the breeds. The underlying physiological reasons are discussed.

Endocrine studies on exotic goats are well documented during oestrous cycle, pregnancy and parturition (Irving *et.al.* 1972 and Umo *et.al.* 1976). However, scanty studies are reported on endocrine status of indigenous goats during estrous cycle (Pathak *et.al.* 1990) and around parturition (Jain *et.al.* 1980). Hence, the present studies were undertaken to study the trend of progesterone profiles around parturition in Surti and Marwari goats.

Materials And Methods

The pluriparous goats, each of Surti and Marwari breeds were selected from departmental farm. Blood samples were collected once daily during 135 days of gestation till parturition and at interval of two days between parturition till 20 days post partum. Serum was separated and stored at -20°C after adding 0.01 per cent merthiolate as a preservative till analysed. Serum progesterone was estimated by Radio Immuno Assay (Kubasik, 1984) with some modifications.

The data were analysed to know the variations due to stages of pregnancy as well as due to animal in individual breed with the help of Randomised block design. Difference between breeds and stages around parturition were tested statistically using student 't' test (Steel and Torrie, 1960).

Results And Discussion

Plasma progesterone concentration measured during antepartum, on the day of parturition and post-partum stages revealed that the level of progesterone in both the breeds of goats was decreasing steadily from day 7 antepartum to day of parturition with slight elevation here and there (Table 1). These results are in agreement with that reported by Umo *et.al.* (1976) and Jain *et.al.* (1980) in goats. Level on the day of parturition (Surti -1.30 ng/ml, Marwari -1.40 ng/ml) declined significantly (Table 2) in both the goat breeds. This is to facilitate easy parturition as per the progesterone block theory of Caspo (1956) and Irving *et.al.* (1982). Similar results have been observed by Fylling (1970) in ewes.

After parturition, the level started declining considerably coming to base level by day 6 post partum, in Surti (0.66 ± 0.39 ng/ml) and by day 8 post partum in Marwari goats (0.52 ± 0.25 ng/ml). This decline can be explained as degeneration of pregnancy corpus luteum, because in goats the ovaries are the main source of progesterone in pregnancy (Meites *et.al.* 1951). However, considerable amount of progesterone was available during earlier days of post-partum periods. This can be attributed to leaching of hormone from deposits in fatty tissue (Hopman *et.al.* 1979).

There after, on day 8 pp in Surti and day 10 pp in Marwari, the level started increasing. This can be explained as ovarian activity which might have started by this time and the source of progesterone may be from small follicles, lining development of follicle or granulosa cells of the ovary (Short, 1962 and Webb *et.al.* 1980).

Present studies indicate that the blood progesterone profiles of Surti and Marwari goats around parturition follow the trend similar to exotic goats, cattle and buffaloes and provide the understanding of ovarian recyclicity during post partum period which in Surti starts as early as day 8 post partum and in Marwari day 10 post partum, with initiation of follicular development.

Table 1 : Serum Progesterone (ng/ml) around parturition in Surti and Marwari goats (Mean ± S.E.)

Stages of pregenancy	Surti goats (n = 10)	Marwari goats (n = 10)
A. ANTE-PARTUM		
7 days	9.30 ± 0.86^{ab}	9.18 ± 0.87^{ab}
6 days	9.88 ± 0.62^{a}	10.42 ± 0.47^{a}
5 days	9.24 ± 0.98^{ab}	8.54 ± 0.72^{b}
4 days	9.26 ± 0.75^{ab}	9.23 ± 0.42^{ab}
3 days	8.21 ± 0.81^{b}	8.63 ± 0.83^{b}
2 days	6.55 ± 0.53^{c}	6.56 ± 0.83^{c}
1 day	3.23 ± 0.39^{d}	2.88 ± 0.36^{d}
B. PARTURITION	1.30 ± 0.53^{e}	1.40 ± 0.35^{e}
C. POST PARTUM		
2 days	0.68 ± 0.21^{e}	1.39 ± 0.44^{e}
4 days	0.71 ± 0.26^{e}	0.48 ± 0.21^{e}
6 days	0.66 ± 0.39^{e}	0.56 ± 0.17^{e}
8 days	1.03 ± 0.41^{e}	0.52 ± 0.25^{e}
10 days	0.79 ± 0.29^{e}	1.43 ± 0.42^{e}
12 days	1.39 ± 0.49^{e}	0.86 ± 0.21^{e}
14 days	0.99 ± 0.32^{e}	0.43 ± 0.11^{e}
16 days	0.47 ± 0.18^{e}	0.71 ± 0.30^{e}
18 days	0.87 ± 0.48^{e}	0.54 ± 0.16^{e}
20 days	0.46 ± 0.18^{e}	0.40 ± 0.21^{e}

N.B. : Values with different superscripts differ significantly with each other at 5% level of significance.

Table 2 : Analysis of variance for serum progesterone around parturition in Surti and Marwari goats.

Source	d.f.	Mean sum of square	
		Surti	Marwari
Stage	17	147.62^{**}	150.73^{**}
Animal	9	5.58^{*}	4.78^{**}
Error	153	2.502	2.11

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

REFERENCES

Caspo, A. (1956). Progesterone 'block'. Am. J. Anat. **98**:273-291.

Fylling, P. (1970). The effect of pregnancy, ovariectomy and parturition on plasma progesterone level in sheep Acta. Endocrinol, Copenhagan, **65**:273-283.

Hopman, B., Wagner, W.C., Hison, J.E. and Bhar, J. (1979). Anim. Reprod. Sci. **2**:253.

Irving, G., Jones, D.E. and Kniftom, A. (1972). Progesterone concentration in the peripheral plasma of pregnant goats. J. Endocrinol, **53**:447-452.

Jain, G.C., Batra, S.K., Pahwa, G.S. and Pandey, R.S. (1980). Plasma progesterone level during late pregnancy in goats Zbl, Vet. Med. A. **27**:513-516.

Kubasik, N.F. (1984). Evaluation of a direct solid phase RIA for progesterone. Clin. Chem. **30**:284-286.

Meites, J., Webster, H.D., Young, F.W., Thorp, F. and Hatch, R.N. (1951). Effects of corpora lutea removal and replacement with progesterone on pregnancy on goats. J. Anim. Sci. **10**:411-416.

Pathak, M.M., Patel, A.V., Jaiswal, R.S. and Mehta, V.M. (1990). Circulating levels ofprogesterone and estrogen in cycling goats. Ind. J. Anim. Sci. **60(7)**, under publication.

Short, R.V. (1962). J. Reprod. Fertil. **4**:27. Cited by Jain, G.C. and Pandey, R.S. (1987). Circulating levels of progesterone and PGF_2 during oestrous cycle in buffalo heifers. Ind. J. Anim. Reprod. **8(2)**:116-117.

Steel, R.G.D. and Torrie, J.H. (1960). Principles and procedures of statistics. Pub. McGraw Hill Book Co., Inc., New York, U.S.A.

Umo, I., Fitzpatrick, R.J. and Ward, W.R. (1976). Parturition in the goat : Plasma concentration of prostaglandin F. and steroid hormones and uterine activity during late pregnancy and parturition. J. Endocrinol, **68**:383-389.

Webb, R., Lamming, G.E., Hynes, N.B. and Foxcroft, G.R. (1980). Plasma progesterone and gonadotropin concentration and ovarian activity in post partum dairy cows. J. Reprod. Fertil. **59**:133-134.

IJAR:11:2:155-157:1990

Estimation Of Daily Sperm Production in Cross-bred Bucks Using Depletion Trials

METILDA JOSEPH and K. PRABHAKARAN NAIR

College of Veterinary and Animal Sciences, Mannuthy-680 651 Kerala.

ABSTRACT

The daily sperm production in cross-bred bucks was estimated by depletion trials. The number of services to exhaustion and the total ejaculate volume (ml) per day ranged from 6-10 and 2.6-10 respectively. Daily sperm production (x 10^6) was found to be 4861.30.

* * *

There are several reports on the estimation of daily sperm production (DSP) in bulls, buffalo-bulls and in rams using depletion trials (Boyd and Van Demark, 1957; Ortavant, 1958; Amann and Almquist, 1962; Sengar and Sharma, 1965). Such information on bucks is not available in literature and hence this study.

Materials And Methods

The procedure of Boyd and Van Demark (1957) was followed with partial modification. Six healthy Alpine-Saanen-Malabari cross-bred bucks aged 2-3 years were

* Forms a part of the M.V.Sc. Thesis by the Senior author.

utilized for the study. Within a three hour period a maximum of 10 ejaculates were collected from each buck in rapid succession using A.V. for goats, at intervals of 1, 4 and 7 days following an initial exhaustion with a maximum of 10 ejaculates. The ejaculates collected on each day from each buck were pooled to find out the total ejaculates volume. The spermatozoan concentration of pooled samples was estimated using photo--electric calorimeter and total spermatozoa in each depletion was worked out. The DSP was worked out using regression of time interval on sperm count (Snedecor and Cochran, 1967).

Results And Discussion

The number of collections for depletion and total ejaculate volume per day (ml) ranged from 6-10 and 2.6-10 respectively. The total ejaculated sperms (x 10^6) in successive depletions from the bucks at different time intervals is presented in Table 1.

Treating sperm numbers as dependent variable and time interval as independent variable, regression equation was worked out as follows :

Y = 3593.32 + 1267.98 X

Y = 3593.32 when X (interval in days) is 0

Y = 4861.30 when X is 1 day

Y stands for sperms in millions

From the regression equation it is seen that each day increase in interval was responsible for an increase of 1267.98 x 10^6 sperm over and above the residual sperm reserve (3593.32 x 10^6) which became available for ejaculation regardless of the interval or extent of depletion. Thus the sperm production per day (x 10^6) estimated for these bucks was 4861.30. Chang (1945) and Ortavant (1958) reported the DSP (x 10^6) in rams to be 8600 and 5500 respectively. But in the present study in goats, DSP is found to be lower than in rams. Joseph and Nair (1988) reported that DSP (x 10^9) in bucks was 3.79 ± 0.20 by testicular homogenate method. The higher values obtained in the present study can be attributed to incomplete depletion of epididymal sperm reserves, as on initial exhaustion many of the bucks gave only upto 6 ejaculates.

Acknowledgement

This paper is published with the permission of the Dean, College of Veterinary and Animal Sciences, Mannuthy. The financial assistance in the form of University Merit Scholarship to the senior author is gratefully acknowledged.

REFERENCES

Amann, R.P. and Almquist, J.O. (1962). Reproductive capacity of dairy bulls. VII. Direct and indirect measurement of testicular sperm production. J. Dairy Sci. **45**:774-80.

Boyd, L.J. and Van Demark, N.L. (1957). Spermatogenic capacity of male Bovine. I. A measurement technique J. Dairy Sci. **40**:68-97.

Chang, M.C. (1945). The sperm production of adult rams in relation to frequency of semen collection. J. Agric. Sci. **35**:243-250.

Joseph, M. and Nair, K.P. (1988). Daily sperm production and epididymal sperm reserves in Alpine-Malabari cross-bred bucks. Indian J. Anim. Reprod. **9**(1):30-32.

Ortavant, R. (1958). Le cycle spermatogenetique Chez le Belier, D.Sc. Thesis, University of Paris, Paris, France.

Sengar, D.P.S. and Sharma, U.D. (1965). Studies on successive semen ejaculates of buffalo bulls. II. sperm reserves and daily sperm production. Indian J. Dairy Sci. **18**:61-64.

Snedecor, G.W. and Cochran, W.G. (1967). Statistical Methods, 6th Ed. Oxform and IBH Pub. Co., New Delhi, pp. 93-289.

Table 1 : Semen volume and total sperm counts of bucks on depletion.

Buck No.	Details of depletion: Initial — Total volume (ml)	Initial — Sperm concentration per ml (x 10^6)	Initial — Total spermatozoa (x 10^6)	One day interval — Total volume (ml)	One day interval — Sperm concentration per ml (x 10^6)	One day interval — Total spermatozoa (x 10^6)
1	4.1	4300	17630	2.7	2680	7236
2	10.2	1560	15912	5.0	1450	7250
3	3.3	2820	9306	2.0	1820	3640
4	9.0	1520	13680	4.0	1500	6000
5	5.4	1900	10260	2.6	1760	4576
6	5.4	1400	7560	4.0	900	3600
Mean	6.23	2250	12391.33	3.38	1685	5383.67
± S.E.	± 2.75	± 1129.73	± 3972.48	± 1.13	± 586.44	± 1683.35
	Four day interval			Seven day interval		
1	3.5	2440	8540	8	2140	17120
2	5.4	1450	7830	10	1520	15200
3	3.9	2300	8970	5.4	2060	11124
4	5.5	1200	6600	9.5	1440	13680
5	5.8	1360	7888	5.6	1560	8658
6	4.6	1280	5888	8.0	1520	12160
Mean	4.78	1671.67	7619.33	7.74	1706.67	12990.33
± S.E.	± 0.94	± 549.05	± 1168.34	± 1.93	± 308.20	± 3011.78

ERRATA

The following errors in IJAR Vol. 11, No. 1, June 1990 may please be corrected. Inconvenience caused is regretted.

No.	Item	Error	Correction
1.	Page 8 Left Col. line 14–15	6·16 ± ·10 Litres	6·16 ± 0·10 Litres
2.	Page 8 Right Col. line 24–25	29% R.H. (Production 5·71 Lit./hr.)	29% R.H. (Production 6·31 Lit./hr.) 31·95°C, 25·30°C and 55% R.H. (Production 5·71 Lit./hr.)

IJAR:11:2:158:1990

Incidence Of Mid-cycle Oestrus In Cattle And Buffaloes.

G.P. ROY, A.P. SINGH, M.H. AKHTAR, K.M. PRASAD, R.B. SINGH and S.N. SINHA

Department of Gynaecology, Obstetrics and Artificial Insemination, Bihar Veterinary College, Patna-800014.

Incidence of mid-cycle oestrus in cattle and buffaloes has been reported by Kavani and Kodagali (1984) and Danell (1987). Since, it may be misleading in proper detection of oestrus in successfully carrying out A.I. programme, the present study was undertaken to record the incidence of mid-cycle oestrus in cattle and buffaloes.

The study was conducted at outdoor clinics of Gynaecology Department where A.I. is carried out routinely since past many years. In all 12,449 cattle and buffaloes were included in the study. Of these, 1,760 were indigenous heifers; 3,021 indigenous cows; 2,516 crossbred heifers and 4,017 crossbred cows. Buffaloes comprised 319 heifers and 816 cows. It was evident from the records and history that these animals had exhibited symptoms of oestrus about 9 to 11 days back. They were evincing symptoms of oestrus at regular interval and were apparently free from infections. Animals were examined for symptoms and signs of oestrus which included swelling of vulva, mucus discharge, vaginal hyperaemia and opening of portio. Main emphasis was given on the palpable findings of the ovaries for the presence of C.L. and ovarian follicle. Contraction, tonicity of the uterus and grading of the cervical mucus crystallisation pattern as per Danell (1984) were also recorded. Animals having large follicle and regressed C.L. on the ovaries were diagnosed to be in standing oestrus, while those having fully developed C.L. co-existing with palpable ovarian follicle (10 mm in diameter) were diagnosed to be cases of mid-oestrus.

It was evident that in all types of animals studied, there was incidence of mid-cycle oestrus which was 62.02% in crossbred cows, 40.98% in indigenous cows, 20.03% in crossbred heifers and 10.97% in indigenous heifers. The incidence of mid-cycle oestrus in buffaloes was found to be 5.01% and 26.10% in heifers and cows respectively. The results of the study are in agreement with two wave growth of ovarian follicles (Rajakoski, 1960; Settergren, 1977 and Danell, 1987).

Acknowledgement

The authors are grateful to Dr. R.N. Singh the then Principal, Bihar Veterinary College, Patna for providing necessary facilities.

REFERENCES

Danell, B., Gopalkumar, N., Nair, M.C.S. and Rajagopalan, K. (1984). Indian J. Anim. Reprod. 5:1-7.

Danell, B. (1987). Ph.D. Thesis, Uppsala, Sweden.

Kavani, F.S. and Kodagali, S.B. (1984). Indian J. Anim. Reprod. 5:38-41.

Rajakoski, E. (1960). Cited by Danell, B. (1987).

Settergren, I. (1977). Personal Communication.

IJAR:11:2:159:1990

Incidence Of Reproductive Disorders In Cattle Of Kashmir

SADAT REHMAN[1], G.M. WANI[2] and Z.A. KHAN[3]

S.K. University of Agricultural Sciences and Technology, Post Box 461, G.P.O. Srinagar-190001, Kashmir.

Reproductive failures result in tremendous economic loss to livestock productivity. According to White and Nicholas (1965), the animals culled in USA on the basis of infertility and sterility were as high as 12-19%. No such information is available from Jammu and Kashmir State. As such the present study was conducted to find out the incidence and various forms of reproductive losses in cattle of Kashmir Valley.

A team of experts visited various animal husbandry units in district Anantnag, Pulwama and Baramullah in the year 1987-88. Infertility camps were organized in collaboration with Animal Husbandry Department. Animals were brought to the camp from different villages on pre-information by the Animal Husbandry Department personnel. The animals were examined by reproduction specialists and the data on various aspects of reproduction were recorded. The data was analysed as per Snedecor and Cochran (1967).

A total of four camps were held. The total number of infertile animals examined were 200.

The overall incidence of infertility was 24.3% (Table 1). The overall incidence of anoestrus (36%) is higher than that reported by White and Nicholas (1965).

Table 1 : Incidence of reporductive failures in Cattle.

S.No.	Condition		Incidence
1	Under developed genitalia		30%
2	Cervicitis		10%
3	Metritis		16%
4	Anoestrus		36%
	i) Anoestrus due to malnutrition	31%	
	ii) Non-specific anoestrus	1%	
	iii) Lactational anoestrus	4%	
5	Silent heat		1%
6	Persistant corpora lutea		7%
7	Over-all incidence		24.3%

REFERENCES

Snedecor, G.W. and Cochran, W.G. (1967). Statistical Methods, 6th Edn., Oxford and IBW Publishing Co., New Delhi.

White, J.M. and Nocholas, J.R. (1965). Reasons for disposal of Pennsylvania Holstein Cattle. J. Dairy Sci. 48(4):512.

1. Asstt. Prof. Department of Extension Education, FVSC and A.H. Shuhama.

2. Professor and Head of the Division.

3. Associate Professor.

IJAR:11:2:160-162:1990

Note On Dicephalic Monster In Surti Buffalo

K.M. PANCHAL[1], D.M. BHAYANI[2], SWETA PANDYA[3], A.S. BAGI[4], M.T. PANCHAL[5] and H.J. DERASARI[6]

College of Veterinary Science and Animal Husbandry, Gujarat Agricultural University,

Anand Campus, Anand-388 001 (Gujarat)

Monstrocity denotes the abnormal anatomy of the fetus caused by the chromosomal aberration or due to unfriendly environmental conditions in womb of the mother and consequent trauma to the developing embryo, resulting in an abnormal appearance.

The case reported is a female monster of Surti buffalo. The dead calf was removed by a forceful traction at full term pregnancy. The buffalo was bred by A.I. at the Veterinary Clinics of the college.

Externally the calf was having two heads, two necks, common back, two loins and two crop regions with a pair of pectoral limbs and two pairs of pelvic limbs. A rudimentary fore limb of about 5 cm in length with a hoof and a subcutaneous rudimentary bone was found to be attached on dorso-lateral aspect of the right partner of the fetus (Fig.1). On detailed dissection from the ventral aspect of thorax and abdomen, viscera were present in the common thoracic and two separate abdominal cavities which were divided by a common partition of diaphragm.

Fig. 1 : Gross photograph of Dicephalic monster

Digestive system : All the organs of this system were double in number right from mouth to anus. The liver was on cranio-ventral aspect of the abdomen, as an irregular shaped single organ with many lobes and larger in size. The gall bladder was single and located on visceral aspect of the ventral lobe (Fig.2). It was leading to a single bile duct opening into duodenum of left side fetus. The pancreas was single in loop of duodenum of either side, with a pancreatic duct opening into it. The spleen of the left fetus was

1, 2, 5 and 6. Assistant Professor
3. Vety. Officer,
4. Associate Professor.

elliptical in shape and it was found normal in development, while that of the right fetus was rudimentary, oval shaped and nearly half in length than that of left fetus.

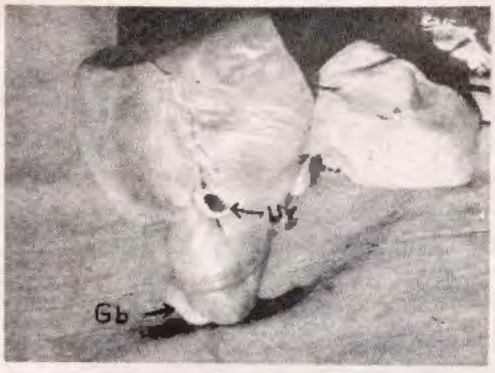


Fig. 2 : Liver of Dicephalic monster showing irregular shape with prominent Umbilical vein (U_v) and the position of gall bladder (G_b).

The deciduous inciors were yet not erupted, while all the premolars were erupted in both foeti. The salivary glands were normal in 3 pairs for each head.

Respiratory system : Organs including thymus and thyroid glands were also paired.

Circulatory system : The heart was a paired organ covered by a common pericardial sac, of which the left heart was completely developed with all four chambers. Between two atria of the left heart, a large foramen ovale was persistent. The interior of all chambers in left heart was normal in development. The apex of the heart as a whole covered by a common pericardium, was formed by a left ventricle of the left heart. While, the right heart was rudimentary without discernible atria and ventricles, with a blunt apex. The inter-ventricular septum was absent in the right heart, forming a common narrow space and the wall of the same was very much thick (Fig. 3).

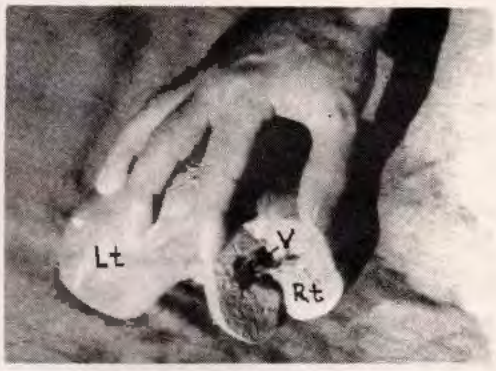


Fig. 3 : Conjoined heart of Dicephalic monster showing normal left heart (L_t) and rudimentary right heart (R_t) with a common narrow ventricular space (V).

The aorta of the left heart originated from the left ventricle. After giving a thick common brachio-cephalic trunk, it passed backward and upward in a common thoracic cavity, pierced through the left side of diaphragm into the abdomen. The abdominal aorta of the left fetus gave an equal sized unusual branch which curved transversely to the right sided fetus and united with its small abdominal aorta. The vasculature from the right heart was poorly developed, with haphazard branches.

There was a ligamentous connection between the two hearts, which was made up of descending branches of coronary vessels covered by connective tissue when it was incised. It showed that the vascular supply to the right heart may be from the coronary artery of the left heart as the right heart was rudimentary.

Urinary system : It also comprised of double set of organs with normal development. The kidneys, ureters, urinary bladder and urethra were paired. Urachus coming from either

side of a bladder was entering into a common umbilical cord which was continuous into a common placenta.

Genital system: The feti were of female sex and both of them were having a separate pelvic cavity. The ovaries were rudimentary, while the tubular organs were normal in development with broad ligament and mesovarium. The external genitalia in both the foeti were also normal. The accessory female sex glands were normal on either side.

IJAR:11:2:162:1990

A Note On Congenital Face Malformation In A Sheep Foetus

A. MARU, C.P. SRIVASTAVA, A.K. MATHUR[1] and S.C. DUBEY

Division of Animal Health, Central Sheep and Wool Research Institute, Avikangar-304501 Rajasthan

On 20th April, 1989 a case of dystokia was reported in a (mutton type) sheep. The head of the foetus was hanging outside the vulva. Dystokia was relieved after correction and manual traction of the dead foetus. On examination the full term foetus appeared to have a bull dog like face without eyes. Only a depression was seen at the site of the right eye. The jaw was with poor development of oral muscles prohibiting normal opening of the mouth cavity. There was absence of nasal orifices and the forehead was bulging. Foetal development beyond neck appeared normal.

Teratogenic role of blue tongue virus, a disease endemic in the Institute's flocks may be a probable reason for the deformity. The dam was found to be a sero reactor though it had no history of clinical blue tongue disease during the gestation period.

Fig.1 : A sheep foetus with bull dog like face malformation.

Acknowledgement

Authors thank the Director, CSWRI for the facilities and Dr. B.S. Mehta for presenting the case.

1. Senior Scientist, Division of Animal Reproduction.

IJAR:11:2:163:1990

Use Of Dexamethasone In The Expulsion Of Mummified Foetuses In Swine

T. GOGOI, K.C. DEKA[1], D. KALITA and K.C. NATH[1]

All India Co-ordinated Research Project on Pigs, Assam Agricultural University, Khanapara, Guwahati-781022.

Foetal mummification is a common problem in pigs and one or more foetuses may be found mummified at the time of farrowing. Four gilts maintained at the AICRP on Pigs, AAU, Khanapara, Guwahati failed to farrow on the expected date of farrowing. It was observed that the udder and vulva of all four gilts regressed to normal non-gravid size within one month after the expected date of farrowing.

Each of the four gilts was injected with 12 mg. of dexamethasone (Doxona Vet Cadila, Ahmedabad-380008) intramuscularly on two alternate days. The animals were then observed for signs of farrowing and with the help of a vaginal speculum, the cervix was examined. On the sixth day of treatment, the cervix was found open with some dirty discharge in three gilts. These three gilts expelled mummified foetuses and the foetal membranes on eighth day of the treatment. The gilt which failed to farrow after treatment was found to be non-pregnant at slaughter. It appeared that, dexamethasone, commonly used in large farm animals for induction of parturition (Adams, 1969; Lauderdale, 1972; Wagner *et.al.* 1974; Sloss and Dufty, 1980), expulsion of mummified foetuses and in hydramnios (Sloss and Dufty, 1980) could be effectively used in pigs for expulsion of mummified foetuses. Tamuli *et.al.* (1988) also reported similar effects of dexamethasone in the bitch.

REFERENCES

Adams, W.M. (1969). The Elective Induction of Labour and Parturition in Cattle J. Am. Vet. Med. Assoc. **154**:261.

Lauderdale J.W. (1972). Effect of Corticoid Administration in Bovine Pregnancy, J. Am. Vet. Med. Assoc. **160**:867.

Sloss V. and Dufty J.H. (1980). Hand Book of Bovine Obstetrics Pub. William and Willkins, Baltimore/London.

Tamuli M.K.; Rajkonwar C.K. and Borgohain B.N. (1988). Dexamethasone an Answer to Incomplete Abortion in Bitch, Indian Vet. J. **65**:935.

Wagner W.C., Willham R.L. and Evans L.S. (1974). Controlled Parturition in Cattle, J. Anim. Sci. **38**:485.

1. Department of Gynaecology, Obstetrics and Artificial Insemination.

IJAR:11:2:164-167:1990

Comparative Efficacy Of Prostaglandin F_2 Alpha With Two Dose Rates And Routes Of Administration In Subestrus Buffaloes*

A.P. GAUTAM, K.G. KHARCHE[1] and M.S. THAKUR[2]

Department of Obstetrics and Gynaecology, Veterinary College, Jabalpur-482001 (M.P.)

ABSTRACT

Twenty eight subestrus buffaloes randomly divided equally in four subgroups were treated with Dinofertin (PGF_2 alpha) at two dose rates (5 and 10 mg) by intra-uterine and intra-vulvo-submucosal routes of administration. In 5 mg PGF_2 alpha treatment groups, 92.86% buffaloes exhibited estrus at 95 to 218 hours of treatment, majority of them in weak estrus and the C.R. was 40%, whereas in 10 mg PGF_2 alpha treatment group, 100% buffaloes exhibited estrus at 75 to 162 hours of treatment with 42.86% C.R. and majority of them were in intermediate and intense estrus indicating that estrus intensity increased with higher dose level. However, both the routes of administration (I.U. and IVSM) gave more or less, similar response without any significant difference.

* * *

Optimal reproduction is the yardstick of successful Livestock Husbandry. Subestrus or silent estrus constitutes the single largest factor responsible for poor reproduction in buffaloes. One of the effective methods of overcoming the problems associated with inherent difficulties of heat detection in buffaloes is by carrying out experimental studies with hormonal agents such as PGF_2 alpha which will not only induce heat but also bring about successful ovulation. Although PGF_2 alpha has been used to control estrous cycle in buffaloes, the success and costs involved have been far from reassuring due to high dose (25 mg IM) recommended for proper luteolytic response. However, intrauterine administration reduces the dose to 5-10 mg, though it presents difficulty in passing of pipettes. Evaluation of alternative route of administration such as intra-vulvo-submucosal may combine economy, convenience and efficiency. Hence, the present investigation was designed to evaluate the comparative efficacy of PGF_2 alpha with two dose rates (5 and 10 mg) by I.U. and IVSM routes of administration.

Materials And Methods

The present investigation was done on 35 buffaloes maintained at composite Livestock Farm, Adhartal. Buffaloes (5 to 10 years of age) with history of pre and post service anestrus and having palpable C.L. without apparent infection of genital tract were selected for the study. Such 35 selected

* Part of M.V.Sc. and A.H. (Obst. and Gynae.). Thesis submitted by the Senior author to Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P.)

1 Head, Department. 2. Assistant Professor

(7 normal cycling and 28 subestrus) buffaloes were divided randomly in three groups (one control and two treatments). The buffaloes of subgroups IIa and IIb were treated with 5 and 10 mg of PGF_2 alpha respectively, by intrauterine infusion and of subgroups III a and III b were treated with the same dose rate by intra-vulvasubmucosal route on day 6 and 16 of estrous cycle after confirming the presence of C.L. (Table 1).

Following treatment, all animals were closely observed daily morning and evening for estrus response. Teaser bull was also used to detect estrus. Buffaloes responding to treatment as well as those of the control group showing normal heats were given natural services. They were examined per rectum on day 10 post estrus to detect C.L. indicative of ovulation at induced heat. Pregnancy was confirmed 60 days after service by gynaeco-clinical examination. The response of PGF_2 alpha (Dinofertin) was noted between two dose rates (5 and 10 mg) and routes (I.U. and IVSM) on estrus response, estrus induction time, type of estrus and number of animals conceived in induced estrus (Table 1).

Results And Discussion

In 5 mg PGF_2 alpha treatment groups (IIa and IIIa), out of 14 buffaloes, 92.86% exhibited estrus between 95 to 218 hours with estrus intensity as 0% intense, 46.15% intermediate and 53.85% weak. Estrus response was detected by external visual symptoms in 38.46% and by teaser bull in 61.54% buffaloes with C.R. of 40%. These observations are in close agreement with those of Chauhan *et.al.* (1982) who recorded 74% estrus response and 44% fertility rate with 5 mg PGF_2 alpha administration I.U. in buffaloes and also with the reports of Rao and Rao (1979) who found 94.4% estrus response and 44.4% fertility with 500 mg cloprostenal (synthetic analogue of PGF_2 alpha) by IVSM route in 36 subestrus buffaloes. Singh *et.al.* (1987) treated 16 subestrus buffaloes with 6 mg of PGF_2 alpha by IVSM route and found 81.25% estrus response within 7 days of treatment with C.R. of 53.84%. Present investigation indicates that the buffaloes treated with 5 mg PGF_2 alpha showed weak and intermediate estrus response only and this dose probably was not sufficient to induce intense estrus, with a comparatively longer induction time.

In 10 mg PGF_2 alpha treatment groups (IIb and IIIb), 100% buffaloes exhibited estrus at 75 to 162 hours with 35.71% intense, 42.86% intermediate and 21.43% weak estruses. Oestrus response was detected by external visual symptoms in 50% and by teaser bull in the other 50% buffaloes. C.R. was 42.86%. Prasad *et.al.* (1979a) and Chauhan *et.al.* (1982) observed 72.72% and 71% estrus response within 2 to 4 days with 53.84 and 44% C.R., respectively, with 30 mg of PGF_2 alpha given by I.M. route. Likewise Singh *et.al.* (1979), Khurana *et.al* (1981), Khurana and Gupta (1982) and Dhaliwal (1985) observed similar estrus response with 25 mg of PGF_2 alpha by I.M. route. These observations approximate with the present findings indicating that estrus response with 10 mg PGF_2 alpha by I.U. or IVSM route was more or less similar to that of 25-30 mg of PGF_2 alpha by I.M. route or natural estrus in normal cycling buffaloes. Thus, 10 mg dose may be considered reliable for inducing estrus in subestrus buffaloes reducing the cost of PGF_2 alpha therapy considerably.

Table 1 : Comparative efficacy of prostaglandin F_2 alpha with two dose rates and routes of administration in subestrus buffaloes.

Comparative parameter	Group	No. of buffaloes responding to treatment out of 14	Estrus induction time (hrs)	Types of estrus			Estrus detection by		Conception rate
				Intense	Intermediate	Weak	External visual symptoms	Teaser bull	
Control	I Normal cycling	Control	Control	3(42.86)	2(28.57)	2(28.57)	4(57.14)	3(42.86)	57.14
Dose									
5 mg	IIa and IIIa (Subestrus)	13(92.86)	95-218	0(0.00)	6(46.15)	7(53.85)	5(38.46)	8(61.54)	4(40.00)
10 mg	IIb and IIIb (Subestrus)	14(100.00)	75-162	5(35.71)	6(42.86)	3(21.43)	7(50.00)	7(50.00)	6(42.86)
Route									
Intrauterine	IIa and IIb (Subestrus)	14(100.00)	87-216	2(14.28)	6(42.86)	6(42.86)	5(35.71)	9(64.28)	5(41.86)
Intravulvo-submucosal	IVa and IIIb (Subestrus)	13(92.86)	75-218	3(23.07)	6(46.15)	4(30.77)	7(53.84)	6(46.15)	5(41.66)

Figures in parentheses indicate per cent observations.

In I.U. treatment group (IIa and IIb), there was 100% estrus response, 87 to 216 hrs. estrus induction time, estrus intensity : 14.28% intense, 42.86% intermediate and 42.86% weak, 35.71% estrus detected by external visual symptoms and 64.28% by teaser bull with C.R. 42.66%. The corresponding values for IVSM treatment groups (IIIa and IIIb) were 92.86%, 75 to 218 hours, 23.07% intense, 46.15% intermediate, 30.77% weak, 53.84%, 46.15% and C.R. 41.66% respectively. These observations showed non-significant differences in estrus response by both the routes. But I.U. therapy can be handled only by experienced person to avoid genital injury. Therefore, the IVSM route is preferable because of easy administration with almost similar response.

In the present investigation, conception rates with 5 mg and 10 mg dose rates were almost similar. Woollen (1984) also reported that the CRs for treated and control cows were equal indicating that PGF_2 alpha treatment did not enhance or decrease fertility. As indicated earlier, 10 mg PGF_2 alpha by I/VSM route is better for induction of estrus in sub-estrus buffaloes.

REFERENCES

Chuhan, F.S., Sharma, R.D. and Singh, G.B. (1982). Responses of different doses of prostaglandin F_2 alpha on estrus induction fertility and progesterone levels in subestrus buffaloes. Theriogenology, **17**:247-253.

Dhaliwal, G.S. (1985). Clinico-biochemical studies on prostaglandin induced estrus in buffaloes using two routes of administration. M.V.Sc. Thesis. Punjab Agricultural University, Ludhiana (Punjab).

Khurana, H.K. and Gupta, R.C. (1982). Note on the use in prostaglandin F_2 alpha for induction of estrus in buffalo heifers. Indian J. Anim. Sci. **52**(9):806-808.

Khurana, N.K.; Tyagi, R.P.S.; Gupta, R.C. and Verma, S.K. (1981). Use of PGF_2 alpha for induction of estrus in subestrus buffaloes (Babalus bubalis) Indian J. Expt. Biol. **19**(17):653-655 (Cited by Dhaliwal, 1985).

Prasad, A.; Bachlaus, N.K.; Arora, R.C. and Pandey, R.S. (1979). Synchronization of estrus in buffalo heifers with prostaglandin F_2 alpha (Tham salt) and fertility on A.I. with Frozen Semen. Indian J. Exp. Biol. **17**:416-417.

Rao, A.V.N. and Rao, S.V. (1979). Treatment of subestrus buffaloes with cloprostenol. Vet. Rec. **105**:168-169.

Singh, G.B.; Dugwekar, Y.G.; Sharma, R.D.; Chauhan, F.S. and Singh, M. (1979). Treatment of subestrus buffaloes with prostaglandin F_2 alpha Vet. Rec. **104**:412-413.

Singh, G.; Singh, G.B. and Sharma, R.D. (1987). Ovulation and fertility after an inter-vulvo-submucos injection of prostaglandin F_2 alpha in subestrus buffaloes Indian J. Dairy Sci. **11**(2):324-325 (Anim. Breed. Abstr. **56**(6):3562).

Woollen, T.S. (1984). Cloprostenal may be the needed ingredient to reduce calving intervals. Vet. Med. Small Anim. Cli. **79**(1):1312-1316 (Cited by Dhaliwal, 1985).

IJAR:11:2:168-169:1990

Use Of Lutalyse* For Bovine Foetal Mummification

S. BALASUBRAMANIAN[1], T.G. DEVANATHAN, A. SUBRAMANIAN and V.N. SESHAGIRI

Department of Obstetrics and Gynaecology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Madras - 600 007.

ABSTRACT

A case of mummification of foetus in a crossbred Jersey heifer and its successful treatment with lutalyse injection is reported.

* * *

Bovine foetal mummification occurs in the third to eighth month of gestation, but most commonly during middle trimester of pregnancy. The etiology of foetal mummification is impossible to determine because the time of foetal death is not known and it is difficult to determine the causative agent for the autolysis and mummification of foetus and its membranes (Roberts, 1971).

Case History

A two years old cross bred Jersey heifer reported to be six month pregnant was admitted in the Madras Veterinary College Clinic with the history of passing dark brownish red discharge from the vagina for the last 2 days and was off-feed.

Clinical Examination : Rectal examination revealed fairly thick uterine wall and absence of cotyledons, but the conceptus alone without foetal fluids and fremitus could be palpated. The case was diagnosed as foetal mummification and treated.

Treatment: Lutalyse 25 mg. was administered as a single intramuscular injection on 6.2.90 at 9.25 AM. The animal was kept under observation for 24 hrs. after the injection. Mummified foetus was expelled at 8.00 PM. on the same day. The probable stage of gestation of the expelled foetus was assessed by measuring the crown-rump (CR) length of the foetus. Terramycin liquid 20 ml. with 30 ml. of distilled water was infused intrauterine to prevent infection. The animal made an uneventful recovery.

Discussion

Foetal mummification has been treated less reliably with oestrogen preparations earlier (Roberts, 1971) and the use of large doses of glucocorticoids to cause expulsion has not been reported (Adams, 1969). With the advent of prostaglandins, the above approaches have lost importance mainly due to their less precision and reliability. Since mummification is usually characterised by a persistent Corpus Luteum it can be treated with PGF_2 alpha preparations.

Talbot and Hafs (1974) and Jackson and Cooper (1977) reported that the expulsion of mummified foetus is brought about in 96-120 hrs. after the injection, which is due to luteolytic effect of prostaglandin. In the present study, the foetus was expelled within 24 hrs. after Lutalyse injection.

Acknowledgements

The authors are thankful to the Dean, Madras Veterinary College, Madras for providing the necessary facilities.

* Lutalyse (Dinoprost Tromethamine) Up John Co.

1. Assistant Professor

REFERENCES

Adams, W.M. (1969). The elective induction of labour and parturition in cattle. J.A.V.M.A. **154**(3):261.

Jackson, P.S. and Cooper, M.J. (1977). The use of cloprostenol for the termination of pregnancy and the expulsion of mummified foetus in cattle. Vet. Rec. **100**:361-363.

Roberts, S.J. (1971). Veterinary Obstetrics and Genital Diseases. 2nd Edn. CBS Publishers and Distributors, India.

Talbot, A.C. and Hafs, H.D. (1974). Termination of bovine pregnancy complicated by mummified foetus. Vet. Rec. **95**:512.

IJAR:11:2:169-170:1990

Uterine Abscess In A Repeat Breeder Cow

N.C. SHARMA, G.R. SINGH, C.S. CELLY, A.K. ARORA and J.S. SANDHU.

Indian Veterinary Research Institute, Izatnagar-243122 (U.P.)

ABSTRACT

A rare case of uterine abscess in a repeat breeder cow, its surgical removal, post-operative recovery resulting in pregnancy is reported.

* * *

Case History

A six year old cow was brought to the A.I. centre, with the history of repeat breeding for the last 20 months. Earlier she had delivered one calf. The cow was served six times naturally and eight times with A.I. During the period the cow was treated severally for repeat breeding condition without any success.

Examination and Diagnosis : The body condition of the cow was good. Gynaeco-clinical examination was carried out. The ovaries were found to be normal and free from ovaro-brusal adhesions. There was a C.L. in left ovary. The uterus on palpation revealed a hard, tense and firm mass 3" in diameter inside the lower third of the left horn. The case was diagnosed as having either a tumor or a long standing abscess with thick walls. Finally it was decided to undertake laparotomy to remove the contents.

Laparotomy was performed in standing position under local anesthesia. Abdominal cavity was approached through vertical incision in the paralumber fossa. Uterus was then pulled gently and a hard swelling at the lower third of the left horn was located. Exploratory puncture of the hard swelling revealed the presence of thick pus (Fig 1). It was then incised and the pus drained out. After evacuation, the abscess cavity was thoroughly cleaned with oxytetracycline. It was then sutured with continuous lamberts sutures using chromic catgut No. 2. The abdominal wound was sutured in layers in routine manner. Local and parenteral antibiotic coverage was given.

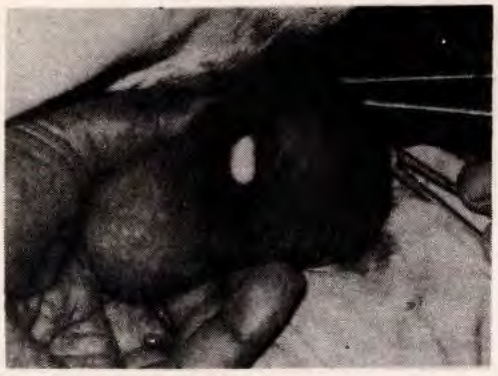


Fig. 1 : Uterine abscess with thick pus.

Bacteriological examination of the abscess contents: The uterine abscess contents were collected in a sterilized tube. Bacteriological examination of the pus revealed the presence of corynebacterium pyogenes. As per the recent literature it is listed under group G streptococcus.

Results And Discussion

Sutures were removed after seven days and the healing was uneventful. After 28 days of the operation the cow exhibited estrus with normal and clear discharge. Sexual rest was given for one cycle. During thenext heat, which was normal cyclic, she was inseminated. Pregnancy was confirmed after 2 months.

The repeat breeding condition in this cow may be due to infection. The source of infection may be either from the bull semen at the time of natural service or A.I. Failure of earlier intrauterine treatments may either be due to low dose or non-specificity of drugs as a result of which organisms must have developed resistance and set up low grade infection of the endometrium. It is likely that the uterine wall might have been injured either at the time of A.I. or intrauterine infusions and the infection localised in the uterine wall resulting in abscess. The abscess appears to be of long duration as revealed by hard thick fibrosed walls of the abscess. This abscess resulted in partial occlusion of the uterine cavity. The cow was repeating possibly because of early embryonic deaths. This condition is allied to Roberts (1971) who reported that uterine abscess may be a sequel to a severe metritis or injury to the uterus due to improper or rough use of an inseminating pipette especially in infected uterus, and the cow may fail to conceive. Sharma *et.al.* (1978) stressed the importance of drug sensitivity test of the uterine infectious agents for repeat breeding cases before adopting any intrauterine treatment.

REFERENCES

Roberts, S.J. (1971). Veterinary obstetrics and genital diseases. Pub. Scientific book agency, Calcutta.

Sharma, N.C., Kulshrestha, S.B., Kaushick, S.N. and Katpatal, B.G. (1978). Study of repeat breeder cows and their treatment with special reference to drug sensitivity of infectious agents. Indian J. Anim. Sci. 48:153.

IJAR:11:2:171-172:1990

Unique Case Of Utero-rectal Fistula In Buffalo

P.K. SHRIVASTAVA[1], R.L. SHIRALI[2] and D.D. HERANJAL[3]

Animal Disease Research Laboratory, National Dairy Development Board, Anand-388 001.

Utero-rectal fistula is not reported in any species. A very rare case of utero-rectal fistula caused by rupture of dorsal wall of uterus and ventral wall of rectum due to excessive movements and pressure of pointed bones of macerated foetus in a buffalo is reported here.

* * *

Case Report

A Surti buffalo aged 8 years in second lactation was presented with a history of faeces passing through the vagina. On rectal palpation, a macerated foetus was felt which was removed. The animal did not respond to the routine medical treatment and was emaciating constantly. Hence it was euthanised and postmortem was carried out suspecting recto-vaginal fistula. On opening the carcass, extensive adhesions of uterine wall with peritonium, large intenstine and omentum were seen. The right horn of uterus revealed a thick walled fibrous fistula connected with the rectum, which contained 11 pieces of long, sharp pointed and several small bones of macerated foetus embedded in loose faecal material along with a 2.5" long piece of hay (Fig. 1). The uterus, cervix and vaginal walls were found soiled with faeces, evidently leaking from the rectum through the fistula. The right ovary revealed persistent corpus luteum.

Discussion

Hunshamar *et.al.* (1981) reported the incorrect insemination procedure as a cause of uterine rupture. In this case, it appeared that the rupture of the dorsal wall of right horn of the uterus and ventral wall of the rectum was caused by the pressure of catheter and protruding pointed bones of macerated foetus. The foetal bones were pressed into rectum probably during repeated recumbancy causing pressure on the uterus. The protruding bones caused continuous irritation which resulted in fibrosis of the fistular wall and the chronic peritonitis was perhaps the reason for the extensive adhesions in the peritoneum, large intestine and omentum.

Tamponing with Lugol's iodine and several gynaceo-clinical examinations would have further aggravated the situation. Robert (1986) observed some faeces and a mucopurulent discharge from vulva in chronic cases of rupture of dorsal wall of vagina and ventral wall of rectum. Our observation of the fecal discharge from vulva is in agreement with Robert (1986), though muco-purulent discharge was not noticed.

REFERENCES

Hunshamar, R., Haaland, M., Kvammen, A., Mjolsnes, O. and Oma, P. (1981). Norsk Veterinaerlidsskriff **92**:519-23 (Vet. Bull **51**:3522).

Roberts, S.J. (1986). Veterinary Obstetrics and Genital Diseases. pp 357-359. 3rd Edn. Wood stock, Vermont 05091.

IJAR:11:2:172:1990

Uterine Torsion In A Goat

C. CHANDRASHASAN, A. SUBRAMANIAN and K. KULASEKAR

Veterinary College and Research Institute, Namakkal-637002, Tamil Nadu.

Torsion of the uterus is not uncommon in dairy cattle, but occasionally observed in goats. Roberts (1971) reported that the incidence of uterine torsion in dairy Cattle was 7.3% Sane *et.al.* (1982) reported a high incidence of uterine torsion in buffaloes ranging from 22.53% to 60.30% and in cattle ranges from 5% to 33% of total cases of dystocia recorded. Vyas (1987) reported a case of uterine torsion in a goat.

A case of uterine torsion in a goat was treated at the Veterinary College Clinic, Namakkal. The torsion was of 180° towards the right side. Caesarean Section was performed as the attempt to detort the uterus by Schaffer's method was futile. The recovery was uneventful.

REFERENCES

Roberts, S.J. (1971). Veterinary Obstetrics and genital diseases (Theriogenology) II Edition, CBS Publishers and distributors, India.

Sane, C.R., Luktuke, S.N., Kaikini, A.S., Hukeri, V.B., Deshpande, B.R., Velhankar, D.P., and Kodagali, S.B., (1982): Reproduction in farm Animals (Theriogenology) Varghese Publishing House, Bombay-400 014.

Vyas, U.V. (1987). Uterine Torsion in a goat. Indian J. of Anim. Reprod. **8**:62.

ISSAR News

Dr. Asim Bala, M.V.Sc., Ph.D. Member of Parliament

Dr. Asim Bala, M.P.

Dr. Asim Bala is elected as a Member of Parliament in the 9th Lok Sabha election held on 24th October, 1989 from Nabadwip Constituency of Nadia District, West Bengal on the Communist Party of India (Marxist) ticket winning by a margin of over 52,000 votes.

He had a distinguished career working in various capacities as Assistant Animal Husbandary Commissioner, Govt. of India and later as Academician in Vidyasagar University, Midnapore and subsequently in Bidhan Chandra Krishi Vishwavidyalaya, Mohanpur (Nadia), West Bengal. He was Vice-President of the West Bengal Veterinary Association and is presently working for Indian Veterinary Association.

It is indeed a matter of great pride for we Indian Veterinarians that Dr. Asim Bala is the champion of Veterinary Profession in Lok Sabha. He readily responded to our request and his message to IJAR is published elsewhere in this issue.

We congratulate Dr. Asim Bala and wish him all success in his noble task of serving our country.

• • •

Dr. S.K. Verma, Professor of Veterinary Gynaecology, HAU, Hissar, visited Egypt under Indo-ARE Work Plan from 10-4-90 to 3-5-90. During his visit, Dr. Verma delivered lectures and rendered Expert advise.

We coongratulate him on his assignment and wish him many more laurels in future.

• •

Dr. B.K. Bhavsar Joint Director of Animal Husbandary, Gujarat State, Ahmedabad is the new President, ISSAR Gujarat Chapter. Dr. B.M. Bhat and Dr. B.A. Odedara are Co-Presidents. Dr. J.H. Prabhakar of NDDB is Hon. Secretary; Dr. S.H. Parekh is Joint Hon. Secretary and Dr. H.J. Derashri is Treasurer of Gujarat Chapter. There are 16 Executive Committee Members and Dr. S.B. Kodagali is the Patron of this Chapter.

We wish this dynamic team a grand success.

• • •

A one day seminar on 'Hormonal Control of Reproduction in Bovines' was organised by ISSAR Gujarat Chapter on 8th September 1990 at Himatnagar. It was sponsored by the Sabarkantha District Co-operative Milk Producers Union Ltd.

Shri Kemabhai H. Patel, Chairman, Sabarkantha Milk Union inaugurated this Seminar. Dr. C.H. Joshi, Director of Animal Husbandry Gujarat State was the Chief Guest. Technical

lectures were delivered by eminent scientists Dr. Y.G. Dugwekar, Dr. V.M. Mehta and Dr. H.J. Derashri. Slide show on Reproductive Pathology was presented by Dr. J.H. Prabhakar, Senior Executive (RC), NDDB, Anand.

Over 120 Veterinary Officers participated in the Seminar which was a grand success.

• • •

National Symposium on 'Recent Biotechnical Advances in Animal Reproduction' and the 9th Annual Convention of ISSAR, organised by ISSAR; HAU Hissar and ICAR New Delhi will be held at HAU, Hissar from 6th to 8th February 1991.

Scientists participating in the Symposium are requested to ensure that their Registration Forms/Delegation Fees reach Dr. S.K. Khar, Organising Secretary, Prof. and Head, Deptt. of Gynaecology and Obstetrics, College of Veterinary Sciences, Haryana Agricultural University, Hissar, **by Monday 31st December 1990,** positively.

FROM SECRETARY'S DESK

Dear Member,

Through my appeal/circular letters addressed to different State Chapter Secretaries in the country, I tried to focus your attention towards entries to be forwarded for consideration in respect of Professor Lagerlof Memorial Award, Dr. G.B. Singh memorial awards, ISSAR fellowship award and Best Chapter awards (1989). I am happy to communicate you that the response received for various awards was very encouraging except that for Best Chapter award. Various award committees have been constituted and all the entries received have already been forwarded to respective Chairman/Members of the Committee for critical evaluation. I am expecting their evalution reports very soon. The names of winners of various awards will be communicated to you all at the inaugural function of our Society's ensuing Symposium to be held at H.A.U., Hissar, 6th to 8th February, 1991.

I hope that all the life members of our Society have received the intimation regarding holding of our Symposium at HAU, Hissar, 6th to 8th February, 1991. I am once again approaching all the Vice-Chancellors of Agricultural Universities, Directors of Department of Veterinary and Animal Husbandry, Directors of National Laboratories, Directors of Co-operative Milk Societies etc. with a request to depute maximum number of Scientists/Officers for the ensuing National Symposium.

I am happy to inform you that I have succeeded in procuring financial assistance from the Indian Council of Agricultural Research, New Delhi for publication of our Society's Journal for last three years. I have approached the Council this year also for providing financial assistance for publication of Journal and also holding Soceity's National symposium. I hope to succeed in getting financial assistance like last three years this time also.

May I appeal to you all once again to try to enroll maximum number of annual Subscribers/Life Members/Institutional or sustained members from your respective State and also try to secure/book maxium number of advertisements for our Journal (IJAR) as well as for the Souvenier to be brought out at the ensuing Symposium at Hissar.

Hope to meet many of you in person at Hissar.

With Season's best greetings and wishing you most happy and prosperous New Year.

Yours sincerely
D.R. PARGAONKAR
Secretary, ISSAR

Parbhani
30 OCT., 1990.

Book Review

"Dairy Cattle Production — Selected Readings" 1st Edn. 1990. Pub. BAIF Development Research Foundation, 'Kamadhenu', Senapati Bapat Marg, Pune-411 016 (India). Chapters 21. Pp. 276 Price Rs. 80/- ($18)

This book is a collection of selected topics of practical importance in Dairy Cattle Production. Each of the Chapters is a digest of a particular topic contributed by renowned expertise in the related field of specialization.

Each aspect of dairy cattle production has been dealt with thoroughly extending from Impact of cattle cross-breeding on rural economy; Strategies for germ plasm conservation; Oestrus synchronisation as tool for improved fertility; Repeat breeding in cows etiology and treatment; Production of quality frozen semen; Feeding strategies for high yields and optimum reproductive performance; Fodder from tree species; Newer Drugs for dairy cattle diseases; and Health problems and treatment to Emerging technologies and newer challenges for veterinary profession. There is also an interesting chapter on Application of Homoeopathy in Veterinary field.

A long felt need for compact latest information on Dairy Cattle Production has been met with by this nice book. It is a "must" for all cattle breeders, technical personnel engaged in livestock production and development activities besides teachers, researchers and students of Veterinary and Animal Sciences. It should also adorn the Libraries of Veterinary Colleges, Agricultural Universities, Central Research Institutes, Frozen Semen and A.I. Stations and related Livestock production projects schemes.

Padmashri Dr. Manibhai Desai deserves grateful congratulations for his Dynamic vision and foresight in bringing out this valuable publication.

— A.S. KAIKINI

• • •

"Semen Production and Artificial Insemination" By: Dr. M.R. Bhosrekar. 1st Edn. March 25, 1990. Published by BAIF Development Research Foundation, 'Kamadhenu', Senapati Bapat Marg, Pune-411 016 (India). Pp. 300 Figs. 28, Annexures 4, Price Rs. 80/-

The author Dr. Bhosrekar is a renowned expert authority on Bovine Frozen Semen Technology in India. His vast rich experience in this field of specialization is condensed with uptodate important references in this Book. Besides the basic information, there are several chapters of practical utility, essential for production of quality Frozen Semen. There is also a Chapter on A.I. and Semen production in Goats.

The Chapters on Maintenance, Care and Handling of Frozen Semen Equipment serve as a Ready Reckoner for all technicians involved in Frozen Semen Production and its Field Application. Chapters on Male Infertility, Measure of Reproductive Efficiency, Oestrus Synchronisation and Breeding Management for better fertility are excellent additives to the practical utility of this "matter of fact" Book. Care has been taken to include only the essential vital matter in crisp language making it an interesting reading.

This publication serves as a valuable "Hand Book" on A.I. and Semen Production, ideal for Graduate and Post-Graduate Students of Veterinary Sciences/Animal Reproduction/Animal Sciences, Veterinarians and Live Stock Development Officers, alike. We strongly recommend it for them all, besides Libraries of Agricultural Universities, Colleges of Veterinary and Animal Sciences, Central Institutes and the network of crossbreeding Programmes in our country and abroad.

— A.S. KAIKINI

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.

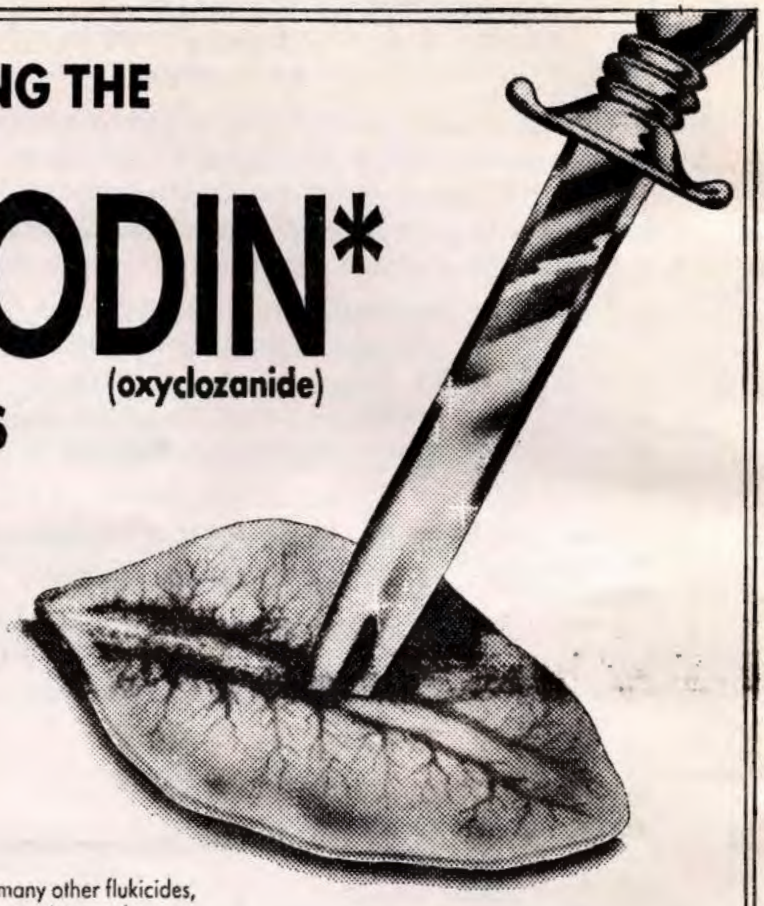
A. RAMAMOHANA RAO
PRESIDENT — ISSAR

ANNOUNCING THE RETURN OF

DISTODIN*

(oxyclozanide)

FLUKE BOLUS & TABLET



Trusted Distodin is back! Unlike many other flukicides, Distodin gives you all the benefits you've sought.

POWERFUL: Distodin kills both mature and immature liver flukes and amphistomes.

Distodin treats both acute as well as chronic fascioliasis.

SAFE: Distodin is safe for weak, young, pregnant or lactating animals.

Distodin may be given together with treatments for other conditions, or with vaccinations.

CONVENIENT: Distodin comes in easy-to-administer and easy-to-measure dosages.

Dose:

Cattle: 1 bolus for 100 kg bodyweight. Calves 1 tablet for 20 kg bodyweight.

Sheep: 1 tablet for 13 kg bodyweight. In the treatment of acute fascioliasis increase the dose 3 times.

Pfizer

Animal Health

DISTODIN*

FLUKES CAN'T WIN AGAINST DISTODIN

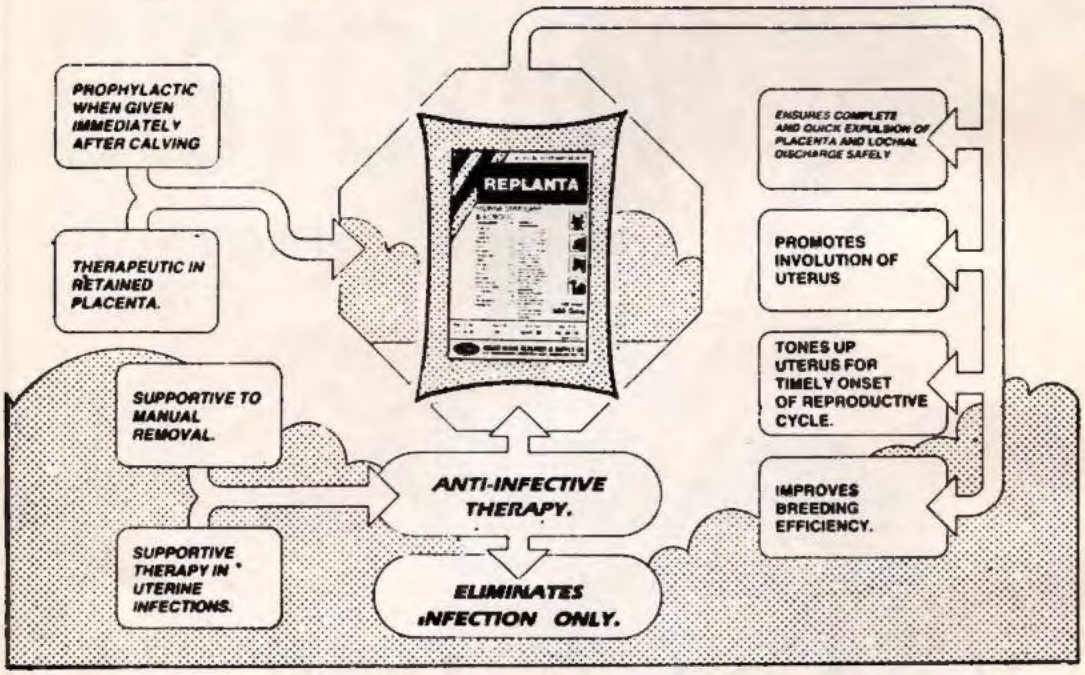
*Trademark of Pfizer Inc., U.S.A. Euphoric Drugs Ltd - Licensed User

pfizer

Manufactured in India by Euphoric Drugs Ltd Express Towers, Nariman Point, Bombay 400 021.

Marketed by PFIZER LIMITED Express Towers, Nariman Point, Bombay 400 021.

MANAGE POST-CALVING EVENTS AND IMPROVE BREEDING EFFICIENCY



REPLANTA

The Classic Herbal Uterine Tonic & Ecbolic

DOSAGE:

Cows & Buffaloes	: 50-60 gms
Mares	: 30-40 gms
Ewes & Does	: 8-12 gms

Administer one double dose orally mixed with jaggery or feed soon after calving and repeat single dose after every six hours till the placenta is shed completely within 24 hours. Continue to administer REPLANTA twice daily for 3-6 days to ensure early involution of uterus, toning up of uterine muscles and improving breeding efficiency.

PRESENTATION: 500 gms

BEING HERBAL, REPLANTA DOES NOT INTERFERE WITH MILK PRODUCTION.

INDIAN IH HERBS

– Nature's way to animal health

Sharda Nagar
Saharanpur-247 001 (UP)

164/3, Vasavi Temple Road
V.V Puram, Bangalore-560 004

NEW BANMINTH (morantel citrate) BOLUS.

PRECISE DOSAGE. NO WASTAGE.

Precise dosage. It's the reason why we changed the size of the most popular deworming bolus. The new Banminth Bolus is just right for 100 kg of animal bodyweight. The weight of dairy animals varies from 100 kg to 500 kg, depending on age, sex and breed. The new Banminth Bolus allows greater flexibility and accuracy of prescribing the specific number of boluses according to bodyweight. Plus you get all the other benefits of Banminth:

- Banminth destroys all major gastrointestinal roundworms – mature and immature – in one single dose.
- Banminth is safe, through all stages of pregnancy.
- With Banminth, milk is safe for human consumption even immediately after deworming.
- Banminth prevents nutritional deficiencies due to roundworms infection, to help improve reproductive efficiency.

Also Banminth Oral Solution is now available in 3% concentration in 500 ml bottles and 5 litre jars.

Dose: 4 ml per 20 kg of bodyweight.

Banminth* (morantel citrate) Forte Bolus

Kills roundworms before roundworms kill profits.

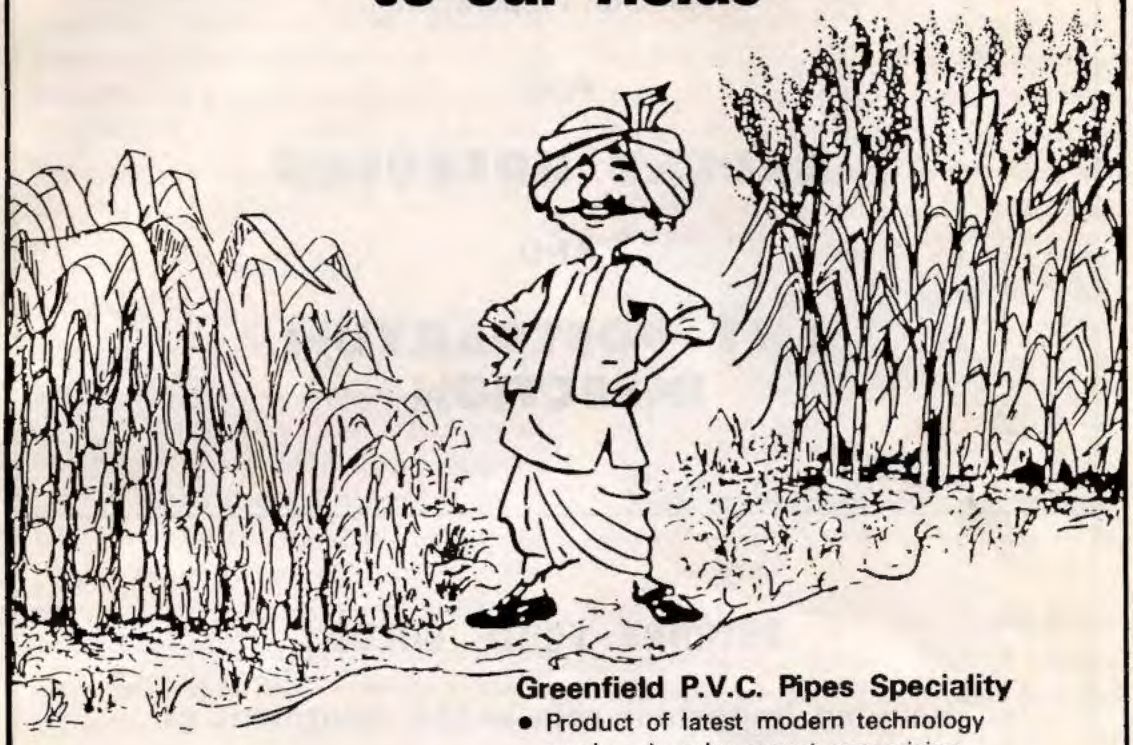
* Trademark of Pfizer Inc., U.S.A.

For further information please write to Pfizer Ltd., P.O. Box No. 11602, Bombay 400 021.

pfizer

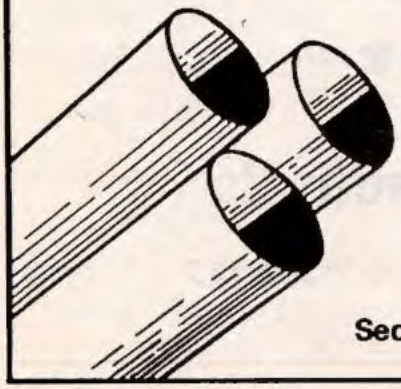
LINTAS BMT P5203

Farmers proudly tell with Joy "Greenfield" brings water to our fields



Greenfield P.V.C. Pipes Speciality

- Product of latest modern technology produced under expert supervision
- Top quality production • Guranteed long life service • ISI Standard certified
- Economic and Durable



Greenfield

GREENFIELD

Chemo-Plast (Pvt.) Ltd.

Office : "Prabha Niwas" Jail Road, Nagpur-440 022
Phone : 25737 Nagpur
Factory: A-37, M.I.D.C. Area, Wardha-442 006
Phone : 2106 Wardha

Secret of Flush Crop Production.....GREENFIELD

DESIGN BY VINOD SAWALKAR NGP-9

METRONIDAZOLE INJ.(VET.)

The Ideal Anaerobicide

YOUR FIRST LINE OF ATTACK IN ANAEROBIC INFECTIONS

Cattle/Buffaloes	Post-partum infections, wound infections.
Horses	Hoof/Frog infections including abscesses and "thrush", wound infections.
Small Animals	Otitis externa, abscesses and wounds, Gingivitis, post-operative sepsis.

Packing Bottle of 100 ml.

RHÔNE-POULENC

MAY & BAKER PHARMACEUTICALS

RHÔNE POULENC (INDIA) LIMITED
RHÔNE POULENC HOUSE WORLI BOMBAY 400 025

Bangalore • Bombay • Calcutta • Guwahati • Hyderabad • Indore • Jaipur • Lucknow • Madras • New Delhi • Patna

SEXUAL HEALTH CONTROL PRODUCTS FROM KARNAVATI

ADIVET	Bolus	: Vitamin A (250000 I.U.) & Vitamin D3 (25000 I.U.)
EUGOLS 2	Liquid	: Lugol's Iodine Solution 2%
EUGOLS 5	Liquid	: Lugols' Iodine Solution 5%
GRISOL	Liquid	: Iodine Solution 2% plus Polyethelene Glycol 10%
ICOC	Bolus	: Cobalt Sulphate (100 mg.), Copper Sulphate (1000 mg.) and Ferrous Sulphate (2500 mg.)
KEPIMIN	Powder	: Mineral mixture with minerals & trace minerals.
TRICIFORTE	Powder	: Cobalt, Copper, Ferrous, Vitamin A, Vitamin D3 and Vitamin E.

Toton Graphics

KARNAVATI PHARMACEUTICALS PVT. LTD. Anil Bhavan, Saijpur Tower, AHMEDABAD 382 345.

RAYMOND'S EMBRYO RESEARCH CENTRE

WORKING WITH TOMORROW'S TECHNOLOGY TODAY

Raymond's Embryo Research Centre undertakes embryo transfer in cows with peerless efficiency and outstanding results To recount—

A. Establishment of E T. pregnancies in 90 recipient cows in one year which includes–
   1. 12 E. T. pregnancies from one donor cow
   2. 11 E. T. pregnancies from another donor cow
   3. 8 E. T. pregnancies from a third donor cow.

B. Birth of 66 E T. calves which included three sets of twins.

C. Birth of 6 calves from frozen embryos.

D. Successful embryo transfer in village conditions at farmer's door.

The Embryo Research Centre now offers a Service Package to you which would comprise–

1. Collection of embryos from your high yielding donor cows snd transferring them in to your recipients.
2. Freezing of embryos.
3. Transfer of frozen embryos.

We also offer frozen embryos from high yielding crossbred Holstein Friesian and pure H. F cows at reasonable price from our Centre.

Please write for more details and service charges to–

Dr. S. G. Zawar,
Manager,
Raymond's Embryo Research Centre,
Gopalnagar–495 663,
Dist : Bilaspur (Madhya Pradesh)

Tel. No. Bilaspur :
4470 / 4220 / 4221 / 4223
Telex No. 0273–215 RCW IN
Fax No. 07752–4612

LIVESTOCK SPECIALITIES

ANALGIN Injection VET — 30 ml Vials
(Highly effective analgesic, antipyretic, antirheumatic agents)

CHLORIL VET Injection — 10 ml, 30 ml vials
(For quick relief from all allergic manifestations)

EPIDOSIN VET Injection — 5 x 5 ml ampules
(For easy expulsion of foetus)

LIVOBEX VET Injection — 10 ml, 30 ml vials
(Liver extract with B-complex Vitamins)

VALGINATE VET Injection — 10 ml, 30 ml vials
(Antispasmodic and analgesic Injection)

Veta-A VET Injection — 5 x 2 ml
(Vitamin A Injection for non-specific infertility)

Robatran VET BOLUS — 5 x 4's
(Co-trimoxazole Bolus)

ROBENDOL VET BOLUS — 5 x 4's
(Mebendazole 500 mg)

TTK PHARMA LIMITED
(Animal Welfare Division)
8 Old Trunk Road, MADRAS - 600 043.INDIA
Quest for Better Health

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.

Printed at Majestic Printing Press, Near Tilak Statue, Mahal, Nagpur-440 002