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Induction of Oestrus and Fertility with CIDR Device and Combination in non Cycling Buffaloes

S.B. ANDURKAR[1] and M.S. KADU[2]

Department of Animal Reproduction (Gynaecology)
Punjabrao Krishi Vidyapeeth, Akola

ABSTRACT

In all 39 non-cycling buffaloes were assigned to short (S-8 days) and long (L-12 days) term therapy either with CIDR (C) implant of proluton depot (P) in combination with prostaglandin (PG) and PMSG. In Buffaloes receiving CIDR treatment, the induction response (Per cent) and the duration for induction (hours) were 100 with 82.28±12.40 in LCPG group, 83.33 with 180.00±82.61 in SCOPG group, 100 with 72.00±12.00 in SCPMS group and 100 with 106.00±39.81 in LC group respectively. Whereas in buffaloes receiving proluton depot in single (LP) and divided (SP) doses, the induction response and duration were 33.33 per cent with 60.00±12.00 hours and 83.33 per cent with 1109.22±367.25 hours respectively revealing significant ($P<0.05$) effect of treatment on induction period.

The intensity of oestrus was better with CIDR implants. The fertility was better in groups receiving long term treatment as compared to those receiving short term therapy. The better feertility in group T 5 was attributed to PMSG used.

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Exogenous administration of progestins for less than 14 days effectively synchronizes oestrus in cattle when combined with either oestradiol at the initiation or prostaglandin administration just prior to progestin withdrawal. Some evidences exist to show that short term progesterone treatment may not result in the same degree of oestrogenic activity as is associated with long term progestagen regimen. Further, there is a growing evidence that PGF 2 alpha has direct effect on the bovine ovary, apart from those of luteolysis, particularly with regards to the post-partum resumption of overian activity. The use of PMSG in combination with progesterone releasing intravaginal device (PRID) in synchronization of heifers (Virakul, 1986) revealed better results in terms of oestrus symptoms and conception rate.

The present investigation envisaged to study the efficacy of CIDR device and other combinations with long and short term therapy in inducing oestrus and fertility in anoestrus buffaloes and to investigate whether CIDR device can be effectively replaced with injectable proluton depot.

MATERIALS AND METHODS

In all 39 healthy, non-cycling pluriparus and pubertal buffaloes were gynaeco-clinically confirmed as anoestrus and grouped so as to include those with similar age and parity in all the experimental groups.

CIDR (Controlled Internal Drug Release) devices are manufactured by Carter Halt Harvey Agricultural Division, Newzealand. The device contains 1.9 gm of micronised U.S.P. grade progesterone. The other drugs used were proluton depot (German Remedies Limited), Folligon (PMSG - Intervet) and Lutalyse (Dinoprost Tromethamine - Upjohn Co.).

All the animals were treated as per schedule and observed for expression of oestrus by parading the approned bull and from behavioural symptoms. Regular Gynaecoclinical examination was carried out daily after cessation of oestrus

1. Livestock Development Officer, Yeotmal (Maharashtra).
2. Dean, Punjabrao Krishi Vidyapeeth, Akola

for ovulatory depression (OVD) or for palpation of CL to decide whether the induced oestrus was ovulatory and pregnancy was confirmed on rectal examination.

RESULTS AND DISCUSSION

Induction response : In groups T 1 and T 6 (with CIDR implant) the induction response was 100 percent the duration for induction was lesser (82.26±12.00 hrs. in group T 1 (receiving PGF_2 alpha) than 106.00±39.81 hours in T6 (Table-2) the difference being non-significant. These findings corroborate with those of Rajamahendran and Thamotharam (1983) and Saini *et al.,* (1988) who reported 100 per cent oestrus response with PRID. Previous studies indicated that treatments for 12 days or less have produced best fertility with concurrent use of either estrogen at the start of treatment or prostaglandin F 2 alpha at or close to the treatment (Smith *et al.,*) (1984).

In group T 4 and T 5, short term progestogen therapy was employed. In group T 4 (SCOPG) out of 6 buffaloes 5 (83.33%) expressed oestrus within av. 180.00±82.61 hours. In group T 5 (SCPMS) out of 6 buffaloes all (100%) expressed oestrus within 72.00±12.00 hours. Thus the use of PMSG in T 5 proved to be slightly superior to the PGF_2 alpha in T 4. Present findings are in accordance with the views that administration of PMSG at the end of shorterm progestogen treatment increased ovulation rate and fertility in cows (Virakul *et al.,* 1986) and McMillan *et al.,* (1989). The use of PGF_2 alpha in group T 4 showed that short term progesterone treatment may not result in the same degree of estrogenic activity as is associated with long term progesterone regimes. Smith *et al.,* (1979) expressed similar views.

In groups (T 2 and T 3) receiving progesterone depot 5(83.33%) and 2 (33.33%) cases responded at a longer interval of 1109.25±367.25 hours and 60.00±12.00 hours respectively. There was a significant ($P<0.05$) difference in induction response and interval between group T 2 and T 3 which indicated that progesterone at divided doses was better as compared to single injection when PGF 2 alpha was given on 6th day The induction response, observed in the various groups in the present study is better than those reported by Rajamahendran and Thamotharam (1983) and Singh *et al.,* (1984).

Length of oestrus (Table 2): The length of induced oestrus in buffaloes treated with CIDR (T 1 and T 6) was (20.85±3.14 and 28.66±8.20 hours), almost similar. However, duration in group T 2 was significantly longer (31.60±7.52 hours) than 20.85±3.14 hours) in group T 1. Similarly, in group T 3 the oestrus was prolonged (44.00±20.00 hours), suggesting that the proluton depot induced longer oestrus. In group T 4 and T 5 the length was 23.20±6.85 and 27.33±6.62 hours respectively. The analysis of variance, revealing significant difference ($P<0.05$). These findings are similar to those reported by Chede (1990).

Of all the buffaloes induced the oestrus was intense in 10 (32.25%) medium in 16 (51.61%) and only 5 (16.12%) expressed weak oestrus. In buffaloes receiving long term treatment with CIDR in group T 1, T 6 and with proluton depot in group T 2, the oestrus was intense in 28.87, 16.6 and 40.00 per cent cases respectively. With short term therapy offered in group T3, T4 and T5, the intensity of oestrus was not uniform, In group T 3 (SPPG) the intensity was medium with very rare expression of behavioural symptoms. The oestrus was intense and medium in 20 and 60 per cent in group T 4 and in 66.66 and 16.66 per cent cases in group T 5 respectively. Thus, in short term therapies probably the combining drug played a role to cause better synchrony and expression of oestrus. These observations are in agreement with Rao *et al.,* (1987) who reported better synchronisation and expression of oestrus in a group receiving PRID for 7 days folowed by 25 mg PGF 2 alpha on the 6th day as compared to other combinations. Further with divided doses of progesterone (proluton depot) probably the Progesterone level is adequately maintained and cause optimum folliculogenesis as compared to the single dose.

Ovulatory status : From 39 treated buffaloes. 31 expressed oestrus and 27 (87.09 per cent)

were ovulatory oestruses. The response was better than reported by Singh *et al.,* (1984). It is evident that the (CIDR) treatment and combinations in the aforesaid study were optimum to induce better development of the dominant follicle to cause ovulation in most of the cases.

Fertility rate (Table 2): The fertility at induced oestrus was 100.00, 71.42 and 66.66 per cent in group T 1, T 6 and T 2, respectively. These observations suggest a better induction response with CIDR over proluton depot. Remaining buffaloes in these groups conceived at the next oestrus taking the fertility percentage to 100.00, 71.42 and 83.33 in respective groups.

With short term therapy in group T 4 and T 5 the fertility was 66.66 and 85.71 per cent respectively. The better fertility in group T 5 can be attributed to PMSG over oestradiol benzoate and prostaglandin employed in group T 4. Better conception rate by using PMSG has also been reported by Virakul *et al.,* (1986) and Rao *et al.,* (1987).

With treatments of proluton depot in divided and single dose and prostaglandin in group T 2 and T 3, the conception rates were 66.66 and 33.33 per cent respectively. The difference is significant ($P<0.05$) and can be attributed to sustained progesterone level and nutritional status of the animals. The fertility observed in all except T 3 group, in the present study, was higher than reported by Saini *et al.,* (1988). McMillan *et al.,* (1989) and Chede (1990) and may be attributed to the better synchrony achieved by the combinations of the exogenous hormones used.

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Table 1. Buffaloes with various treatment regimens under study.

Sr. No.	Group	Code	No.of buffaloes	Treatments
1.	T_1	LCPF	7	CIDR implant for 12 days and PG (Lutalyse) 2.5 ml I/M on day 10
2.	T_2	LPPG	6	Proluton depot 500 mg on 1st and 7th day and PG (Lutalyse) 2.5 ml I/M on day 10.
3.	T_3	SPPG	6	Prolution depot 1000 mg on 1st day and PG (Lutalyse) 2.5 ml I/M on day 8.
4.	T_4	SCOPG	6	CIDR Plus Estrdiol Benzoate for 8 days plus PG (Lutalyse) 2.5 ml I/M. on day 6.
5.	T_5	SCPMS	6	CIDR for 8 days and Folligon (PMSG) 500 I.U. at removal of implant.
6.	T_6	LC	7	Only CIDR implant for 12 days (control) for treatment).

L=Long, S=Short, P=Proluton, C=CIDR
PG= Prostaglandin, PMSG=Pregnant Mare Serum Gonadotrophin
O=Oestrediol benzoate.

Table 2. Effect of treatment of CIDR and various hormonal regimens in Buffaloes.

Group	Total No	Responded %	Duration for induction (hrs)	Length of induced estrus (hrs)	Conception		Fertility %	Services per Conception
					First AI	Second AI		
T1	7	100	82.28±12.40	20.85±3.14	100	—	100	1.00
T2	6	83.33	1109.25±367.25	31.60±7.52	66.66	16.66	83.33	1.20
T3	6	33.33	60.00±12.00	40.00±18.00	33.33	—	33.33	1.00
T4	6	83.33	180.00±82.61	23.20±6.85	50.00	16.66	66.66	1.50
T5	6	100.00	72.00±12.00	27.33±6.62	71.42	14.28	85.71	1.16
T6	6	100.00	106.00±39.81	28.66±8.20	71.42	—	71.42	1.40

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Studies on Blood Serum Levels of Certain Biochemical Constituents in Normal Cycling and Anestrous Crossbred Cows

S.C. VHORA, C.V. DINDORKAR AND A.S. KAIKINI

University Department of Post Graduate Studies
in Gynaecology & Animal Reproduction,
Punjabrao Krishi Vidyapeeth, AKOLA - 444 104.

ABSTRACT

Blood serum levels of cholesterol, total proteins, cobalt, copper and iron were studied in normal cycling and anestrous crossbred cows. In normal cycling cows, cholesterol levels were higher than those in anestrous cows, but the difference was non-significant. Levels of total proteins, cobalt and copper were significantly higher in normal cycling cows than in anestrous cows. Higher levels of Iron were recorded in normal cycling cows. However, the difference between the two groups was non-significant. Possible role of these blood serum constitutents in reproduction is discussed

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Nutrition plays an important role in fertility management in dairy cattle. General health and body weight are usually considered as the guide lines for assessing the nutritional status of the animal. Blood serum levels of various biochemical constituents can be more precise guidelines for correcting reproductive disorders of nutritional origin. Present studies were undertaken to study the levels of certain biochemical constituents in blood serum of normal cycling and anestrous crossbred cows.

MATERIALS AND METHODS

This study was conducted on 12 (six normally cycling and six post partum anestrous) crossbred cows of cattle Breeding Farm, Borgaon, Manju, P.K.V. Akola. Normal cycling cows were selected after thorough gynaecoclinical examination. Cows which failed to exhibit estrus 60 days or more post-partum (P.P.) with palpably smooth inactive ovaries were considered as P.P. anestrous cases.

About 15 ml blood was collected from each cow through jugular vein in big test tube and allowed to clot for separation of serum which subsequently was used for estimation of biochemical constituents.

For estimation of cholesterol and total proteins, "Span Diagnostic Kits" were used. For estimation of trace elements (Co, Cu, and Fe) the serum samples were diluted five fold with triple glass distilled water and estimation done by Atomic Absorption spectro-photometer. All results were directly obtained in concentration units.

RESULTS AND DISCUSSION

The results are summarised in the table. Results revealed no significant difference in the cholestrol level between normal and anestrous cows. On the contrary Kavani *et al.,* (1987) and Pal *et al.,* (1991) reported significant higher levels of cholesterol in normal cycling cows than in anestrous cows. Velhankar (1973) reported a positive corelation between blood cholesterol concentration and energy status of the animal. The energy status of the cows studied was probably the same and hence no significant difference inserum cholesterol level was observed between the two groups.

The average serum total proteins levels were significantly higher in normal cycling cows (8.62±0.13 g / 100 ml) than in anestrous cows (6.82±0.40 g / 100 ml). These findings are in agreement with those earlier reported by Naidu and Rao (1982) and Chetty and Rao (1986). The role of proteins in reproduction of ruminants is equivocal, since some of the published reports indicate that feeding excess proteins results in

lowered fertility while others indicate that deficiency of protein may cause reproductive disturbances in animals. According to Bearclen and Fuquay (1992) diets deficient in protein have resulted in weak expression of estrus, cessation of estrus, repeat breeding etc. However, total proteins in circulation represent a balance between the biosynthesis and catabolism or mechanical loss (Ghosh *et al.*, (1991). The lower level of serum proteins may cause deficiency of certain amino-acids required for the synthesis of gonadotropins, thereby causing reproductive disturbances.

Significant higher levels of serum cobalt were recorded in normal cycling cows (96.67±8.06 mcg / 100 ml) than in anestrous cows (56.67±6.69 mcg / 100 ml). Significant higher levels of serum cobalt in normal cycling cows suggested the importance of cobalt in normal reproduction. Alderman (1963) and Morrow (1980) have reported that cobalt plays a vital role in metabolism of copper, enhancing bovine fertility particularly by feeding combination of copper and cobalt as feed additives.

The average serum copper levels were significantly higher in normal cycling cows (104.17±3.76 mcg / 100 ml) than in anestrous cows (73.33±3.35 mcg / 100 ml). These findings are in agreement with Debas *et al.*, (1987) who recorded higher serum copper level in normal cycling than anestrous cows. Sharma *et al.*, (1988) and Shrivastava (1990) also recorded higher levels of serum copper in normal cycling heifers than in infertile / delayed pubertal heifers. Deficiency of copper is mainly manifested as reproductive disorder in the females.

The average serum iron levels were higher in normal cycling cows (191.67±8.75 mcg / 100 ml) than those in case of anestrous cows (175.831±7.71 mcg / 100 ml). These findings are in agreement with Sharma *et al.*, (1988) who recorded higher serum iron levels in normal cycling vis-a-vis primary infertile Kankrej heifers. Iron deficiency may cause anaemia and secondary inanition which may be reflected as anestrus in cows and delayed puberty in heifers.

Table 1. Average values of certain biochemical Constituents in Blood Serum of Normal Cycling and Anestrous Cross-bred cows.

SR. NO.	Group of Cows	Cholesterol (mg/100 ml)	Total proteins (g/100 ml)	Co (mcg/100ml)	Cu (mcg/100ml)	Fe (mcg/100ml)
1.	Normal Cycling Cows	194.94 ±7.79	8.62 ±0.13	96.67 ±8.06	104.17 ±3.76	191.67 ±8.75
2.	Anestrous Cows	194.16 ±16.54	6.82 ±0.40	56.67 ±6.69	73.33 ±3.35	175.83 ±7.71

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Biochemical Blood Profile of Normally Cycling and PGF_2 Alpha Treated Subestrous Crossbred Cows

AKLANK JAIN AND N.N. PANDITA

Department of Animal Husbandry and Dairying
Institute of Agricultural Sciences, BHU, Varanasi

ABSTRACT

Current investigations were aimed to assess the effects of PGF_2 alpha treatment on the concentration of blood constituents viz;protein, cholesterol, glucose, calcium and inorganic phosphorus. Results obtained elicited no significant impact of the treatment under reference on protein, calcium and inorganic phosphorus of blood. However under subestrus conditions animals revealed a significantly lower concentration of inorganic phosphorus compared to values recorded in samples from normally cycling animals. Blood glucose registered a significant increase during oestrus irrespective of the situation whether animals were normally cycling or treated. Similar was the trend observed in cholesterol concentration the differences in values being significant.

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Earlier finding (Sane, 1977) reveal that some blood constituents play role in the abnormal oestrus cycling of cows. Authentic estimations of the levels of protein, cholesterol, glucose, calcium and inorganic phosphorus may serve as complementary aids to determin the functional status of pituitary gland. To affirm the role of concentration of these biochemical constituents of blood under subestrus condition of crossbred cows prior to and after the treatment by PGF^2 alpha constituted the theme of current investigations.

MATERIALS AND METHODS

Investigations were carried out on crossbred cows selected from the dairy unit of Banars Hindu University. Animals were divided in two groups viz., the normally cycling (Group A) and subestrus cows (Group B) - the latter having been induced to oestrus through administration of 25 mg of Prostaglandin F_2 alpha. Blood samples of the experimental animals were procured for biochemical analysis before oestrus (T1) and at estrus (T2) in group A and before treatment (T3) and at estrus (T4) in group B. Procedure, not withstanding, samples from the control group were collected during non-oestrus phase as well (4 days in anticipation of oestrus) and reckened as day - O.Animals were inseminated in both the groups as per am : pm rule. Rectal palpation was carried out to detect probable oestrus and consequently ovulation was confirmed by locating mature corpus luteum on day - 10. post insemination.

The biochemical parameters as referred to above were studied by following specific method viz., GOD-POD for glucose, Modified Biuret and Dumas method for total protein, Wybenga and Pillegi method, and for cholesterol, calcium and phosphorus as the method described by Gommari (1942) was used.

RESULTS AND DISCUSSION

The concentration of total blood protein and Calcium under different treatment groups prior to estrus and at the time of estrus failed to evince any significant differences within treatments and the control. (Table 1).

Interestingly however, a significant difference ($P<0.05$) in the blood cholesterol level in the normally cycling cows prior to oestrus (T_1) and at the time of oestrus (T_2) was recorded. Identical trend of observations was seen between (T_3) (before treatment) and T_4 (At oestrus) in group

* **Present address:** Dr. Aklank Jain, Asstt. Professor, College of Vety. Sci. & A.H., Jabalpur (M.P.)

B animals that were subjected to PGF_2 alpha treatment. The concentration of cholesterol (169.98±15.47 mg / 100ml) in the normally cycling cows prior to oestrus (T_1) recorded a quantum jump subsequently on the day of oestrus (T_2) registering a value of 249.22±31.06 mg / 100ml. Parallel figures of cholesterol concentration in the treated groups. i.e., T_3 and T_4 were 161.02±13.58 and 235.35±28.29 mg / 100 ml respectively. (Table - 1).

The higher serum cholesterol ($P<0.05$) content of oestrus in both the groups as observed during current investigations stand in agreement with the findings of Purohit and Kohli (1977). Specific opinion of Henericks *et al.,* (1971) in this regard purports to the view that the highest adrenal cholesterol values occur at oestrus when females are under estrogen dominance eventually facing a decline later on when the progesteron phase sets in.

The mean plasma glucose concentration for different treatment groups i.e., T_1, T_2, T_3, and T_4 was 44.86±3.73, 67.69 ±5.00, 37.75 ±3.50 and 57.57±5.07 mg / 100 ml respectively involving a significant difference ($P<0.01$) in glucose concentration in the normally cycling cows prior to and at the time of oestrus. A similar trend ($P<0.01$) was discernible consequent to the treatment by PGF_2 alpha in group B.These observations bear a parity with the earlier thinking by, Sharma *et al.,* (1984) and Agrawal *et al.,* (1985)

Current observation indicating lower levels of glucose under subestrus conditions and thereby leading to the failure of females to exhibit symptom of heat do affirm the findings of Randel (1990) who concluded that glucose concentration modulates reproduction within a specific threshold level.No advantage however, is likely to be served if these levels exceed the threshold status. It might infact lead to a negative impact in case these levels fall below the threshold concentration.

Observations revealed a comparatively higher serum inorganic phosphorus content in T_1 and T2 (5.10±0.20 and 5.47±0.34 mg per cent) in normally cycling as against the PGF_2 alpha induced cows viz., T_3 and T_4 which presented values of 4.02±0.20 and 5.26±0.21 mg per cent, indicating significant differences ($P<0.01$) in the levels of this constituent, from normally cycling (T_1) and the subestrus (T_3) cows. Such however was not the case within T_1 and T_2 or T_3 and T_4

The lower level of inorganic phosphorus under subestrus animals confirms the findings of Beoze (1964). Further, support to this view is lend by Sane *et al.,* (1977) who pointed out that inorganic phosphorus deficiency usually results in deceptive phenotypic oestrus symptoms which even the vasectomised bulls fail to detect.

Table 1. Biochemical Constituents in Normally Cycling and PGF_2 Alpha Treated Subestrus Crossbred Cows.

	Normally Cycling cows (A)		PGF_2 alpha treated cows (B)		
	Before estrus T_1	At estrus T_2	Before treatment T_3	At estrus T_4	'F' value
Total blood protein	7.82± 0.33	8.42± 0.30	7.52± 0.11	7.92± 0.06	2.01^{NS}
Serum cholestrol	169.98± 15.47	249.22± 31.06	161.02± 13.58	235.35± 28.29	3.14^{**}
Blood glucose	44.86± 3.73	67.96± 5.00	37.75± 3.50	57.57± 5.05	13.82^{**}
Blood calcium	7.86± 0.78	8.79± 0.92	7.19± 0.94	7.53± 0.86	0.30^{NS}
Blood Inorganic Phosphorus	5.10^{a} 0.20	5.47± 0.34	4.02± 0.20	5.26^{a}± 0.21	0.78^{NS}

* ($P<0.05$) ** ($P<0.01$) a T_1 differ T_3 ($P<0.05$)

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Blood Biochemical Profiles in Normal Cycling and Delayed Pubertal Crossbred Heifers*

O.P. SHRIVASTAVA[1] and M.S. KADU[2]

Department of Gynaecology and Surgery
Punjabrao Krishi Vidyapeeth, Akola - 444104 (M.S.)

ABSTRACT

Significant differences in some biochemical constituents were observed in normal cycling (N.C.) and delayed pubertal (D.P.) crossbred heifers. The mean serum total protein level was significantly ($P<0.01$) higher in N.C. (7.54±0.11) than in D.P. (6.64±0.26 gm per 100 ml) crossbred heifers. The mean serum cholesterol level was significantly ($P<0.01$) higher in N.C. (109.29±1.85) than in D.P. (97.37±2.15 mg per 100 ml) crossbred heifers. The mean level of calcium was higher (non significant) in N.C. (9.46±0.26) than in D.P. (8.95±0.29 mg per 100 ml) crossbred heifers. The mean level of serum inorganic phosphorus was significantly ($P<0.01$) higher in N.C. (6.17±0.07) than in D.P. (5.57±0.14 mg per 100 ml) crossbred heifers.

——x——x——x——

Besides increasing milk production, one of the important aspect of crossbreeding indigenous cattle was to reduce the age of puberty / maturity to harvest the desired advantages. The success achieved in this aspect seems to be questionable. Recent reports, suggest that the age of puberty in crossbreds is showing increasing trend (Baghel, 1988; Deshmukh and Kaikini, 1989). There is a definite role of protein, calcium and phosphorus in attainment of puberty.

The present study was designed to study the levels of serum total protein, cholesterol, calcium and inorganic phosphorus in normal cycling and delayed pubertal crossbred heifers.

MATERIALS AND METHODS

The study was conducted on 48 crossbred heifers (12 normal cycling + 36 delayed pubertal) belonging to livestock instructional Farm Akola and Cattle Breeding Farm Borgaon, Punjabrao Krishi Vidyapeeth, Akola.

The crossbred heifers which had attained adequate body weight (over 200 kg) and age (above 2 years) but failed to exhibit estrus and on gynaeco-clinical examination had both ovaries smooth and inactive with flaccid and atonic uterus were regarded as delayed pubertal. The crossbred heifers having active ovaries with various grades of palpable corpus luteum and exhibited estrus before the age of two years were regarded as normal cycling.

Blood was collected from all heifers on any one day and serum was separated and preserved in a deep freeze at - 20 C tempearture till analyzed. Total protein, cholesterol, calcium and inorganic phosphorus were estimated with the help of kits supplied by Span Diagnostic Private Limited 173-B, New Industrial Estate, Udhana - 394210 (Gujarat).

RESULTS AND DISCUSSION

The mean level of serum total protein was 7.54±0.11 and 6.64±0.26 gm per 100ml in normal cycling and delayed pubertal crossbred heifers, respectively. The level was significantly ($P<0.01$) higher in normal cycling than in delayed pubertal crossbred heifers.

The low plane of nutrition leading to low protein intake and other constituents necessary

* Part of Ph.D. thesis submitted to P.K.V. Akola (M.S.) by the first author.

1. Associate Professor, Department of Obstetrics and Gynaecology, College of Veterinary Science and A.H., Jabalpur - 482001 (M.P.).
2. Dean, PKU, Akola.

to body weight is considered as the cause of delay in the onset of estrous cycles (Roberts, 1971). In the absence of dietary protein the reproductive failure is due to a lack of ovarian hormones. Protein deficiency retards the development of sex organs and may affect subsequent reproductive performance. The lack of protein may reduce the resistance of animal against infectious disease and may indirectly affect reproduction (Arthur, 1975).

Serum cholesterol level was significantly higher in normal cycling (109.29±1.85) than in delayed pubertal (97.37±2.15 mg per 100 ml) crossbred heifers. Observations of Velhankar (1973) indicating a positive correlation between the higher cholesterol concentration and better reproductive performance appears to be justified in view of present findings where a low cholesterol concentration was observed in delayed pubertal heifers. The difference in average serum cholesterol level in normal cycling and delayed pubertal heifers may be due to several factors.

Serum calcium was slightly higher (non significant) in normal cycling (9.46±0.26) than in delayed pubertal (8.95±0.298 mg per 100 ml) crossbred heifers. Similar findings were also recorded by Naidu and Rao (1982) and Dutta *et al.,* (1988). The lower level of serum calcium may be responsible for delay in puberty as calcium dependent mechanisms are involved in steroid biosynthesis in ovaries. Hurely and Doane (1989) stated that stimulation of LH release from pituitary cells involves a calcium dependent mechanism and that LH is not released in the absence of calcium or in the presence of calcium blocking agents. Present findings are thus in accordance with the above hypothesis.

Present study revealed significantly ($P<0.01$) lower serum inorganic phosphorus level in delayed pubertal (5.57±0.14) than in normal cycling (6.17±0.07 mg per 100 ml) heifers. Similar lower levels were recorded by Bhaskaran and Abdulla Khan (1981), Naidu and Rao (1982) and Chetty and Rao (1986) in anestrus heifers / cows as compared to normal cycling heifers / cows.

The delay in sexual maturity and irregularity in estrus due to direct or indirect deficiency of inorganic phosphorus in cattle was observed by Mahadevan (1963). Eltohamy *et al.,* (1989) also suggested that low phosphorus level in serum may be a factor responsible for inducing infertility.

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Serum concentration of Protein-Bound Iodine in Anoestrous Cross-bred heifers.

T. SAHU[1], B.N. MOHANTY[2], D.K.H. RAY[3], D.N. MOHANTY[4], and A.K. BARIK[5]

Department of Gynaecology,
Orissa Veterinary College,
Bhubaneswar - 651 003.

ABSTRACT

Serum samples of 66 true anoestrus heifers were estimated for the Protein-Bound Iodine (PBI) levels. The serum PBI levels were found to be 3.75 ± 0.11 and 5.57 ± 0.15 mog per cent in delayed matured and normal cycling heifers. Sixty one anoestrus heifers having less than 6.20 ± 2.30 mcg per cent of serum PBI were treated with 1 gram of potassium iodide orally daily for 15 days, which resulted in manifestation of oestrus in 24 (39.34 per cent) animals. The serum PBI level of these animals were estimated to be 5.68 ± 0.10 mcg per cent which differed significantly ($p < 0.01$) from the animals those did not respond to treatment.

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The role of nutritional deficiencies including micro nutrients causing infertility in attainment of puberty at late stage has been well documented. Among trace minerals Iodine is the essential nutrient for life and reproduction which is exceptionally low in the sandy soil near the sea probably due to greater rainfall in coastal region. The deficiency of which either in the feed or in the geographical area can cause the endemic form of anoestrus as the been reported by Smith and Aines (1959). The present study was therefore undertaken to assess the PBI level in delayed matured and normal cycling heifers and supplementation of iodine orally to ascertain its therapeutic effect on the onset of puberty.

MATERIALS AND METHODS

A total of 66 cross-bred heifers of more than 18 months of age having normal health with developed genitalia but smooth and quiscent ovaries from the coastal and hilly regions of Orissa constituted the experimental group. Similarly 15 cross-bred Jersey heifers exhibiting normal oestrus cycle with developed genital organs and functional ovaries were taken as control group. Serum collected from these animals were taken for estimation of Protein-Bound Iodine (PBI) by the modified Alkaline Incineration method as reported by Barker *et al.* (1951).

The animals under investigation (61) having less than normal average value of 6.2±2.3 mcg per cent (Lewis and Ralston, 1953) of serum PBI were treated with 1 gram of potassium iodide per animal per day orally for 15 days. Blood samples were collected 15 days post treatment from all the animals and the PBI level in serum was estimated. The animals which did not respond the same treatment was repeated for another 15 days after one month of the first treatment. Blood samples were again collected after second treatment from those treated group which has manifested oestrus and not menifested oestrus for estimation of serum PBI levels. Third successive treatment was conducted in those animals not exhibited oestrus and post treatmtne serum PBI levels in blood were estimated in both the groups. The data thus obtained were analysed statistically as per the method described by Snedecor and Cochran (1967)

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1. V.A.S., Uppalada, Dist.-Gajapati, Orissa, 2. Dean cum Head of the Department, 3. Reader 4. Lecture Gr. I. 5. Lecturer Gr. I., Department of Gynaecology, Orissa Veterinary College, Bhubaneswar - 651 00.

RESULTS AND DISCUSSION

The serum PBI level of delayed matured heifers and normal cycling heifers were estimated to be 3.75±0.11 and 5.57±0.15 mcg per cent with difference being highly significant (P<0.01). This concures with findings of Dabas *et al.* (1987). The significantly low serum PBI level observed in delayed matured heifers is suggestive to be a contributing factor in delay in the onset of maturity due to hypothyroidism. Sane *et al.* (1982) opined that less thyroid activity affects regulation and release of gonadotropic hormones by anterior pituitary.

Oral administration of iodine both to anoestrus heifers and cows resulted in induction of oestrus as reported by Dabas *et al.* (1987) and Ryot *et al.* (1990). Sixty one heifers with mean serum PBI level 3.52±0.05 mcg per cent were supplemented with potassium iodide. The post treatment serum PBI values of both responded and non responded groups with its analysis of variance during first treatment and subsequent treatment regime are presented in Table 1.

In the present study 68.82 per cent animals with their mean serum PBI level of 5.58±0.07 mcg per cent exhibited oestrus on three successive treatments which is lower than those reported by Dabas *et al.* (1987) but is in close agreement with the observation made by Ryot *et al.* (1990). Analysis of variance in the serum PBI level between pre treatment and post first and second treatment revealed a high significant difference (P<0.01), where as the difference between pre treatment and post third treatment values of responded and non responded groups revealed a significant difference (P<0.05). It was observed that the success rate in second and third treatment is lower than that of the first one. This might be due to the reason that most of good animals during first treatment could better utilise the iodine for body requirement.

In the present study treated group recorded a conception rate of 57.14 per cent, while in the normal cycling group the conception rate was 53.33 per cent which is in close agreement with the findings of Ryot *et al.* (1990). The corresponding value of serum PBI level in both the groups were 5.58±0.07 and 5.57±0.15 mcg per cent. Treatment of anoestrus cattle with iodine in form of potassium iodide gave favourable response in 68.88 per cent of animals. Deficiency in iodine and effective treatment of anoestrus heifers with its supplementation to prevent delayed puberty is suggestive of diagnostic importance to clinicians before adoption of any treatment.

Table 1. Pre-treatment and Post-treatment mean Serum PBI values with standard error of Delayed Matured heifers

	Serum PBI concentration (mcg per cent)	
Pre treatment value	**Responded to treatment**	**Not responded to treatment**
3.52±0.05	5.68±0.10 (24)	—
1st Post treatment	5.46±0.17 (10)	4.36±0.10 (37)
2nd Post treatment	5.44±0.14 (8)	4.89±0.13 (27)
3rd Post treatment	—	5.28±0.11 (19)

	Analysis of Variance		
Groups	**1st Treatment**	**2nd Treatment**	**3rd Treatment**
Pre treatment vs Responded to treatment	400.65**	25.54**	4.56*
Pre treatment vs Not responded to treatment	62.41**	10.26**	4.50*
Responded to treatment vs Not responded to treatment	75.67**	5.51*	0.59^{NS}

Figures in parentheses indicate the number of animals
* = P<0.05 ** = P<0.01 NS = Not significant

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Studies on Circulatory levels of Trace Minerals at different Reproductive status in Goat

B.N. BHATTACHARYYA, S.C. TALUKDAR, R.N. BARUAH, K.K. BARUAH (Sr)*
K.K. BARUAH (Jr.) AND ANUBHA BARUAH

Department of Animal Physiology,
College of Veterinary Science,
Assam Agricultural University,
Khanapara, Guwahati - 781 002.

ABSTRACT

The serum concentrations of copper, zinc, and iron during different stages of reproduction i.e. on the day of oestrus, early pregnancy (0-50 days), mid pregnancy (50-100 days), late pregnancy (100-145 days) and non pregnancy were studied in indigenous goats of Assam. These levels varied significantly ($p<0.01$) between different stages of reproduction. The copper concentration was significantly higher on the day of oestrus than the other stages of reproduction. However, the concentration of zinc was significantly higher in late pregnancy but the iron concentration showed lowest value in late pregnancy.

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Efficient reproduction is a prime criteria for the prosperity of livestock industry and reproduction is closely associated with interaction of hormonal and nutritional status of body. The effect of dietary trace minerals on physiological functions in general and reproduction particularly has been reviewed by Hidiroglou (1979). The levels of different mineral changes in different stages of reproduction (Mehta and Gangwar, 1984). But no information is available on the indigenous goats of Assam. Therefore, the present study was undertaken to know the changes of various levels of trace minerals in different stages of reproduction.

MATERIALS AND METHODS

Observations were made on groups of fifteen female indigenous goats of Assam *(Captra hircus* L.) (between 2-3 years of age) in oestrus, early pregnancy (0-50 days), mid pregnancy (50-100 days), late pregnancy (100-145 days) and non-pregnancy states. The animals were maintained as per standard practices of management and feeding. Blood samples were obtained from each of the experimental animal of different groups and serum was seperated. Serum samples were subjected to determination of trace minerals. Copper, zinc and iron were estimated by using atomic absorption spectrophotometer technique. The results were analysed statistically as per Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The mean values of copper, zinc and iron as recorded in serum of goats of different stages of reproduction are presented in table. It was evident that the Cu and Fe levels were highest ($P<0.01$) on the day of oestrus than the other stages of reproduction. However, the concentrations of zinc showed highest values ($P<0.01$) in late pregnancy.

The highest copper concentrations noticed on the day of oestrus might be due to higher concentrations of circulating oestrogens which is in agreement with the findings of Desai *et al.* (1978). Desai *et al.* (1978) also stated that the serum Cu level can be utilized as an indicator of pituitary gonadotrophins and gonadal steriods levels. The administration of oestrdiol or testosterone in humans (Johnson *et al.*, 1959) and of oestrogen or progesterone in rat (Sato and Henkin, 1973) increased Cu level. In this study, pregnant goats shows lower levels of Cu

* Department of Animal Nutrition.

than the nonpregnant ones. This may be due to increased blood volume during pregnancy (Butler, 1963) and moreover, these oscillations can be explained as a demand and subsequent utilization of maternal copper for the development of foetal nervous system (Hidiroglou and Knipfel, 1981).

The higher levels of Zn in pregnant animals than in nonpregnant animals, may be due to the increased concentration of enzyme carbonic anhydrase (Mehta and Gangwar, 1983), of which Zn is an integral component.

The concentrations of Fe showed peak level on the day of oestrus which is probably due to increased output of follicular stimulating hormone (Desai *et al.,* 1982). The Fe concentrations was significantly lower in late pregnancy. This could be explained by the fact that the pregnancy causes an increase in blood volume, total Hb and red cells. However, as the increase in plasma volume is greatest, there is an apparent decrease in Hb and, consequently, in Fe (Soliman and El-amrousi, 1965). The other possible factor is that in late pregnancy there is increased demand of Fe due to requirement of foetus (Tainturier *et al.,* 1984).

Table 1. Levels of Copper, Zinc and Iron during different stages of reproduction in goat.

Reproductive stages	Minerals		
	Copper (ppm)	Zinc (ppm)	Iron (ppm)
On the day of oestrus	$2.84^{a}\pm0.06$	$1.18^{a}\pm0.21$	$4.42^{a}\pm0.16$
Early pregnancy	$1.54^{b}\pm0.11$	$1.80^{b}\pm0.11$	$4.32^{a}\pm0.12$
Mid pregnancy	$1.48^{b}\pm0.09$	$1.86^{b}\pm0.12$	$4.12^{a}\pm0.14$
Late pregnancy	$1.46^{b}\pm0.08$	$1.94^{b}\pm0.08$	$3.12^{b}\pm0.20$
Non-pregnancy	$2.12^{a}\pm0.12$	$1.11^{a}\pm0.09$	$4.16^{a}\pm0.12$

Means bearing different superscripts differ significantly.

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Oestrus Induction using PGF_2 alpha in Crossbred cows with Post partum clinical endometritis

T.C. JACOB*, E. MADHAVAN AND C.P.N. IYER

Department of Animal Reproduction. College of Veterinary and Animal Sciences, Mannuthy, Kerala

ABSTRACT

Forty two Crossbred cows with clinical endometritis were selected for the trial. Group I consisted 21 animals which were kept as untreated controls. Twenty animals belonging to group II were subjected to induction of oestrus by the administration of 25 mg. PGF_2 alpha (Lutalyse) intra muscularly. Average time taken for induction of oestrus in Group II was found to be 58.95 hours. Duration of oestrus varied from 12-24 house (Mean 22.48 hours) and 24-48 hours (Mean 29.98 hours) in Group I and II respectively. The duration of inducted oestrus was of longer duration than that of natural oestrus. Physical changes of the reproductive tract did not show any marked variations between animals of inducted and natural oestrus. The percentage of weak oestrus (14.28 per cent) in the induced group was higher than that of natural oestrus. (9.52 per cent).

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A trial was carried out to study the effect of administration of PGF_2 alpha in the induction of oestrus in cows with clinical Endometritis.

MATERIALS AND METHODS

Materials for the study consisted of 42 cross-bred cows belonging to the livestock farm Mannuthy, attached to the Kerala Agricultural University. These animals were apparently healthy and maintained under identical conditions of feed and management. All these cows did not conceive beyond 45 days post partum and found to have clinical endometritis as evidenced by aberrations of oestrs, abnormal discharge and a large doughy uterus. The selected animals were randomly alloted to the following two groups.

Group I: Twenty one animals were kept as controls without any treatment.

Group II: Twenty one animals were given Lutalyse* to induce heat at 10-12 days of the cycle when they are having a functional corpus luteum.

Animals in both the groups were observed for onset of oestrum, duration of oestrus, changes in the reproductive tract and intensity of oestrus.

RESULTS AND DISCUSSION

All the animals in Group II evinced oestrus after administration of lutalyse. It is noticed that the interval from the administration of the drug to the expression of oestrus ranged from 36 to 120 hours, with an average of 58.95 hours.

Similar observations were also made by Roche (1974). Gupta *et al.* (1978) and Pant *et al.* (1991), who reported that oestrus could be induced by administration of PGF_2 alpha in cycling cows with in 2-4 days of administration. The duration of oestrus varied from 12-24 hours (mean 22.48 hours) and 24-48 hours (mean 29.98 hours) in group I and II respectively. Thus it could be seen that the duration of induced oestrus was of longer duration than that of natural oestrus. This is in contrast to the earlier reports of Elving *et al.* (1975) and Nair and Madhavan (1984) who reported that the duration of induced oestrus did not show marked variation from the normal oestrus in cross-bred cows. Although, there is a pausity of information on the nature and duration of prostaglandin induced oestrus in crossbred animals, Louis *et al.* (1974) reported that the physiological events which follow treatment with PGF_2 alpha were not distinguishable from those which followed

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*Lutalyse (Inj): 5 ml (Upjohn):- Each ml contains Dinoprost Tromethamine eqvivalent to Dinoprost 5 mg.

naturally occurring luteolysis. However, it was further reported that the response of PGF_2 alpha in terms of duration of oestrus and onset of oestrus was reported to be attributed to the ovarian status at the time of PGF_2 alpha administration. In this study all the animals were in early lactation and the variation in duration of oestrus between natural and induced oestrus might be attributed to this as reported by MacMillan (1983) and Fortin *et al.* (1988). The percentage of intense, medium and weak oestrus was 66.66, 23.80 and 9.52 respectively in natural oestrus while the respective values in induced group were 66.66, 19.04 and 14.28. Although, comparable data on the intensity of of induced oestrus are not available, Ginther (1968) and Nair and Madhava (1984) reported a high incidence of weak signs of oestrus when oestrus was induced with PGF_2 alpha and attributed this to a partial luteolysis resulting weak expression of oestrus in animals affected with subclinicl uterine infections. The present study also concurs with the above observations since the percentage of weak oestrus (14.28 per cent)) in the induced group was higher than that of natural oestrus (9.52 per cent). The physiological changes of reproductive tract did not show any variations between animals showing natural oestrus and those in which the oestrus was induced. All the animals in which oestrus was induced showed vulval oedema, congestion of vulal mucosa and sliminess and maximum percentage of the animals showed high tonicity of uterus similar to natural oestrus. This is in full agreement with that of Schultz (1980) and Wenzel (1991), who also reported that the cyclical changes of reproductive tract was not affected by induction of oestrus with PGF_2 alpha and remarked that luteolysis and changes of reproductive tract either occurring naturally or by induction with exogenous PGF_2 alpha are similar in nature.

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Response of subestrus and true anestrus buffaloes to treatment under field condition

R.P. TIWARI[1] AND S.K. GUPTA[2]

Division of Animal Reproduction, IVRI, Izatnagar

A study of response to treatment of cases of subestrus (n=34) and true anestrus (n=19) in field buffaloes has been made. 31 out of 34 subestrus buffaloes treated with prostaglandin F_2 alpha 25 mg came in heat within 2.87 days post treatment and 18 buffaloes conceived at first estrus and 9 buffaloes conceived at second estrus by natural service of a fertile bull. True anestrus buffaloes were treated with PMSG-1500 IU (Folligon, Intervet, Holland) intramuscularly (n = 7), (Arex Lab.), and with combined therapy of calcium (Caldee-12, Wockhardt - 10 ml I/m), Vit. A (prepalin forte-Glaxo - 5 ml I/M) and phosphorus (Tonophosphan-Hoechst 10 ml I/m) at 48 hourly interval three times. The management of cases of subestrus in field buffaloes with prostaglandin F_2 alpha appears possible with a very good response. The results of treatment of true anestrous buffalo were inconclusive.

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The occurrance of subestrus and true anestrus in buffaloes influencing breeding efficiency under field animals is due to suboptimal feeding levels. The management of subsestrus and true anestrus in buffaloes is therefore, of enormous importance especially under field since the farmers are not well informed. This study reports the observations on the management of subestrus and true anestrus in buffaloes by treatment under field condition.

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MATERIALS AND METHODS

Out of 134 lactating buffaloes, 76 buffaloes were identified for the study. All the animals had calved 60 - 90 days prior to examination and had a history of post partum anestrus. Each animal was examined per rectum twice, at 10 days interval and was grouped as subestrus or true anestrus depending on the ovarian status. In subestrus group one of the ovary had either a follicle or a corpus luteum at some stage, accompanied by normal genitalia. In true anestrus group both the ovaries were small, inactive and there was no uterine abnormalities.

Subestrus buffaloes (n=34) were treated with PGF_2 alpha 25 mg (Dinofertin - Alved, Madras) I/m and repeated after 10 day when animal did not respond. Buffaloes with true anestrus were treated with PMSG 1500 IU (Folligon-Intervet, n=7) I/m once; Clomifene citrate 300 mg (Fertivet-Arex Lab, n=6), orally for 5 days; and remaining 6 buffaloes with true anestrus were treated with combined therapy of calcium (Caldee-12, Wockhardt, 10 ml I/m), Vit. A (Prepalin forte, Glaxo 5 ml I/m) and phosphorus (Tonophosphan, Hoechst 10 ml I/m) 48 hourly, repeated thrice.

All the buffaloes treated with PMSG were given natural service at second estrus after treatment.

RESULTS AND DISCUSSION

In this study 57 buffaloes (54.8%) were subestrus while 19 buffaloes (18.27%) showed true anestrus. Among the cases of subestrus treated with prostaglandin, 31 buffaloes out of 34 exhibited estrus within 2.87±0.11 days and 18 conceived to service at first estrus while 9 conceived to service at second estrus after treatmet.

Among true anestrus buffaloes treated with PMSG intramuscularly (n=7), 5 animals came

Address:

1. Asstt. Professor Veterinary College, Anjora, Durg (M.P.). At present PhD. Scholar (Gynae & Obst.) I.V.R.I., Izatnagar.
2. C-279, Rajendra Nagar, Bareilly 243 122 (U.P.)

to estrus within 4.3 days post treatment and 4 conceived at second estrus. 4, out of 6 buffaloes in the fertivet treatment group came to estrus within 12.4 days and 2 buffaloes conceived. In the cases (n=6) treated with combined therapy of calcium, Vit. A and phosphorus 3 buffaloes showed estrus within 17 days and 2 became pregnant.

Subestrus animals when examined per rectum, at 10 days intervals, reveal presence of a corpus luteum on one ovary. A luteolytic agent like PGF_2 alpha can be employed in such subestrus cases to achieve desirable results (Chauhan *et al.,* 1982) together with use of efficient methods of heat detection during post treatment periods. Several studies have shown the use of .25 and 30 mg PGF_2 alpha intramuscularly, 5 mg by intra uterine, 6 mg by intravulvo submucosal routes in subestrus buffaloes (Singh *et al.,* 1978; Chauhan *et al.,* 1984; Nasier and Hussain, 1984; Williams *et al.,* 1984 Dhoble and Gupta, 1987 and Singh *et al.,* 1987) to be satisfactory with acceptable fertility. The present study, under field condition indicated that PGF_2 alpha injection in subestrus buffaloes is most suited in inducing estrus with good pregnancy rate. Higher incidence of true anestrus was reported in buffaloes during post partum period (Chauhan *et al.,* 1984). True anestrus condition may be a result of suppression of FSH release through effect of lactation, nutrition and systemic diseases (Roberts, 1971). No exact cause of true anestrus could be identified in the animals studied. Since the number of true anestrus buffaloes studied was small no definite conclusion could be arrived at with regards to the effective line of treatment.

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Compartive Efficacy of Some Broad Spectrum Antibacterial Medicines in Repeat Breeder Crossbred Cows Under Field Conditions.

M.K. AWASTHI, and R. K. NEMA[1]

Dept. of Veterinary Obstetrics & Gynaecology
College of Veterinary Science & Animal Husbandry
Anjora, Durg - 491001 (M.P.)

ABSTRACT

Total 60 repeat breeder crossbred cows were treated with Gentamicin sulphate, Ampicillin-Cloxacillin combination and Cephalexin at two dose levels. These antibiotics were infused intra-uterine 24 hours post insemination during same estrus to evaluate the efficacy of these medicines in the treatment of repeat breeding. The best results were obtained with Cephalexin where 90% conception rate was achieved at the dose rate of 1.5 gm. and 750 mg. The pregnancy rate with 1 gm. and 500 mg. of Ampicillin-Cloxacillin combination was 70% and 50% respectively. The lowest conception rate of 50% was obtained with 500 mg. and 200 mg. of Gentamicin sulphate.

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Various antibacterial drugs have been tried from time to time to overcome the problem of repeat breeding in cows with variable results. As the majority of cases of repeat breeding is due to low grade bacterial infection (Krishnamurthy 1974, *Gargetal* 1982), the present trial was conducted with the aim to evaluate the efficacy of Gentamicin, Ampicillin-Cloxacillin combination and Cephalexin at two dose levels in repeat breeder crossbred cows under field conditions.

MATERIALS AND METHODS

The trial was carried out on 70 crossbred cows belonging to private organised farms at Durg and Bhilai area. The cows which failed to conceive for three or more than three times either naturally or artificially were included in the trial. All these animals had normal estrous cycle and estrous period without any palpable pathological problem in their reproductive tract. The experimental animals were grouped into three treatment and one control group. Each treatment group was further subdivided in two subgroups, each subgroup comprised of 10 animals each. Thus 3 treatment groups comprised of total 60 animals while control group consisted of 10 repeat breeder cows. Following protocol was adopted for treatment:

Group I (a) Gentamicin sulphate (Gentabio - Vetcare) @ 200mg.
(b) Gentamicin sulphate @500 mg.

Group II (a) Ampicillin-Cloxacillin (Inclox - Brihans) @1 gm.
(b) Ampicillin-Cloxacillin (Ampoxin-Unichem) @500 mg.

Group III (a) Cephalaxin (Cephalaxin - Glaxo) @1.5 gm.
(b) Cephalaxin @750 mg.

Group IV Control group Without any treatment.

Each medicine was dissolved in 25 ml of sterile distilled water and infused intra-uterine 24 hours after second A.I. during same estrus. Post insemination intra-uterine infusion of antibiotic in the same estrus was carried out with the aim to save the time and expenditure on management and feeding. Every animal was gynaeco-clinically examined at the time of estrus and the animal having abnormal cervico-vaginal discharge was deleted from the trial. Each cow

1. Assistant Professor, Dept. of Animal Production & Management, College of Veterinary Science & Animal Husbandry, Anjora, Durg (M.P.).

was inseminated twice, first at mid heat and second 12 hours after first A.I. with good quality frozen semen. Each cow was re-examined per rectally between 8-12 days after insemination. Animals, which did not have palpable corpus luteum on ovary, were deleted from the trial as such cases were of anovulatory estrus. The response to treatment was assessed on the basis of pregnancy diagnosis between day 50-60. The comparative efficacy of each antibacterial medicine was worked out by considering the conception rates of treatment and control group.

RESULTS AND DISCUSSION

The efficacy of antibacterial drugs in relation to conception rate in repeat breeder cows is presented in (table-1). It is evident that group III gave best result which may be attributed to broad spectrum antibacterial action of Cephalaxin and due to recent use of this medicine. It is interesting to note that in both the sub-groups the similar results were obtained indicating that 750 mg of Cephalaxin is enough to get rid off sub clinical uterine infection, if any. There is pausity of availability of information in the literature regarding the efficacy of Cephalaxin in repeat breeder cows. Kelkar (1988) and Skukla and Pandit (1989), reported poor results of Ampicillin (40-42%). However, in the present study, the combination of Ampicillin with Cloxacillin at the dose rate of 1 gm gave 70% conception. Kavani (1984) reported 73% conception with Gentamicin and the similar results were reported by Dabas and Joshi (1984). However, with present trial, the conception rate with both 200 mg and 500 mg dose rate of Gentamicin sulphate was found to be 50% the lowest in the trial. This lower conception rate may be due to frequent use of this medicine under field conditions that might have resulted in the dvelopment of bacterial resistance. Also, the lowest conception rate (50 per cent) with 500 mg of Ampicillin-Cloxacillin combination indicates that it is better to use 1 gm of said medicine for the treatment of repeat breeding. From the above trial it can be concluded that post insemination intra-uterine infusion in the same heat can be preferred in cases where there is absence of mucopurulent or purulent discharge without waiting for next heat in order to reduce the intercalving period and subsequently economic losses can be reduced to minimum.

Table 1. Efficacy of various antibacterial medicines for conception in repeat breeder crossbred cows.

Group		Antibacterial medicine	Dose	Conception rate (%)
I.	(A)	Gentamicin sulphate	200 mg	50
	(B)	Gentamicin sulphate	500 mg	50
II.	(A)	Ampicillin-Cloxacillin	1 gm	70
	(B)	Ampicillin-Cloxacillin	500 mg	50
III.	(A)	Cephalaxin	750 mg	90
	(B)	Cephalaxin	1.5 gm	90
IV.	(A)	Control	—	30

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"Terramycin / LA, (oxytetracycline) Therapy of Peri-Parturient Cows.

S.B. KODAGALI
Emeritus Scientist
Gujarat Agricultural University
Anand Campus, ANAND 388 001

ABSTRACT

A total of 24 HF cross bred cows were involved in the trial with Terramycin / LA therapy during periparturient period.Cows in the treated group received deep I / M injection of Terramycin / LA, Pfizer Ltd. Bombay, a single dose at the rate of 20 mg / kg (1 ml / 10 kg) body weight between 6 hours to 24 hours postparturition. Regular vaginal inspection & rectal palpations were made at fortnightly intervals from postcalving to confirmation of next pregnancy. The study showed that the interval from calving to first observed oestrus, interval from calving to conception, number of services per pregnancy & the predicted ICP & the previous average ICP in the control Vs treated group were : 58.51±4.73 Vs 54.0±2.38; 105.75±16.26 Vs 62.91±3.61; 1.75±0.35 Vs 1.0±0.0 and 385.75±16.26 Vs 342.91±3.60 (days).

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Many disorders of genital system require accurate functional assessment if treatment is to succeed logically. During postpartum almost all cows acquire some uterine infection (Elliot *et al.,* (1968). This delayed the early complete uterine involution. Days required for complete uterine involution had positive significant correlation (r 0.31) with the occurrences of postpartum oestruses. A treatment trial was undertaken with the objective to study the efficacy of meta-phylactic application of Terramycin / LA (injectable solution) Pfizer Ltd., Bombay during periparturient period in cross bred dairy cows with reference to reproductive performance.

MATERIALS AND METHODS

A total of 24 HF cross bred cows belonging to Saint Xaviers' Research Dairy Farm, Mogar were involved in the trial. Cows with known previous calving history were selected and divided alternately between control and treatment group on calving. Cows in the treatment group received deep I / M injection of Terramycin / LA single dose at the rate of 20 mg / kg (1 ml / 10 kg) body weight; dosage divided into two seperate injection sites, between 6 hours to 24 hours post-parturition. Duration of the study was for a full inter-calving period.

Regular vaginal inspection and rectal palpation was performed at fortnightly intervals during the period from postcalving to confirmation of next pregnancy. Evaluations were made and recorded for the completion of uterine involution (UI) according to Studer and Morrow (1980); commencement of postpartum ovarian activity (POA), oestruses, services, fertile services, calving to first oestrus intervals, calving to conception intervals, number of services per pregnancy, general health and other abnormal conditions (Morrow *et al.,* (1966). Information on age (in months) of the cows, number of previous calvings and the body condition scoring (BCS) at calving was evaluated based on a accepted nine category system of BCS (United States, 1989). The predicted intercalving period (ICP) and the average previous ICP (days) were calculated. Data gathered during the period of study was put to statistical analysis (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

Under the present study the interval from calving to first observed oestrus in control vs treated group of cows was 58.51±4.73 Vs 54.0±2.38 days. Oestrus occurred four days early in the treated cows (Table). This difference was not statistically significant.

In treated cows all the 12 animals showed physiologic (rapid & complete) uterine involution by day 30 while as in control cows on day

30 only 7(58.34%) cows showed physiologic UI and in 5 cows (41.66%) there was delayed uterine involution (DUI). This was manifested by asymetry and thickening of uterine walls which persisted beyond 45 days postpartum. Many factors are attributed for DUI. Adequate body condition reserves should be available for energy utilisation at early postpartum. Within the control group the mean BCS of five DUI cows Vs other remaining cows was 4.5 Vs 6.5. This ment a considerable difference and suggested interaction of nutritional factors.

In the treated group of cows the prdicted ICP was significantly reduced when compared to previous averages. In the DUI cows uterine infection of puerperal period continued persisting and according to Bane (1980) these manifested themselves by mainly endometritis or by repeat breeding in clinically normal animals. Nine cows became pregnant in treated group in contrast to 6 cows in control animals by 75 days post partum. This could be due to the effect of Terramycin LA in controlling uterine infection. This could be the reason for the significantly reduced number of days from calving to conception and reduced number of services per conception in treated group in contrast to control animals.

Thanks are due to Authorities of Saint Xaviers' Research Farm, Mogar, for all the help rendered for conduct of the study to Dr. J.V. Solanki, Dept. of Genetics & Animal Breeding, Veterinary College, Anand for the statistical consultation and to all the Farm staff for the help rendered for the study.

Table I. Clinical response and fertility in Control and Treated Animals.

Item	Control Group	Treated group	't' value & significance
Number of cows	12	12	
Calving to first observed oestrus interval (days)	58.51±4.73	54.0±2.38	0.84 NS
Calving to conception interval (days)	105.75±16.26	62.91±3.61	2.572*
Number of servics per pregnancy	1.75±0.35	1.0±0.0	2.143*
ICP predicted (days)	385.75±16.26	342.91±3.60	2.571*
Animals pregnant at different intervals postcalving:(days)	0	2	
50			
75	6	7	
87 & more	6	3	

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Studies on use of Electronic Probe Estrone to Detect Heat in Cows and to Monitor Optimum Time for Insemination.

M.S. HULSURE, D.R. PARGAONKAR, AND S.A. BAKSHI

Department of Gynaecology & Obstetrics College of Veterinary & Animal Sciences,
MAU Parbhani 431 402 Maharashtra.

ABSTRACT

Twelve cows and six heifers of Red Kandhari breed were selected for the experiment. Estrone readings were taken daily in morning and evening hours over a period of two oestrous cycles.

The mean Estrone readings of cows / heifers taken during different stages of oestrous cycle were 127.85±6.10, 88.33±0.70, 134.916±3.88 and 145.50±2.56 during pro-oestrus, oestrus, met-oestrus and di-oestrus respectively. The mean Estrone reading at the time of insemination was 86.50±0.76.

A positive correlation between low Estrone reading and cows / heifers exhibiting heat was observed. The Estrone, eletronic probe may prove beneficial in detecting the cows in heat and also to moniter the optimum time of insemination, as conception rate with first insemination was observed to be 66.67 percent.

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In recent years few workers have studied electrical resistance of fluids of anterior vagina of farm animals by means of electrical probe (Edwards and Levin, 1974 Gartland *et al.,* 1976. Leidl and Stolla, 1976 and Heckman *et al.,* (1979). The electronic probe helps to detect cows cycling normally and to aid in determining when to expect oestrus. It was proposed to undertake studies on use of Estrone to detect oestrus in cows and to moniter optimum time of insemination under Indian conditions.

MATERIALS AND METHODS

Twelve cows and six heifers of Red Kandhari breed were selected for the experiment. Estrone readings (vaginal electrical resistance) were taken in morning (6 AM) and evening (4 PM) hours over a period of two oestrus cycles, by using Estrone TM instrument (Estrogenix, Inc Po. Box 56, Boulder, Colorado USA). It consist of an electronic control unit and veginal probe with probe connector. The veginal probe was inserted in the Vagina of cow / heifer upto cervix and Estrone readings were obtained on screen of Electronic control unit. A vasectomised teaser bull was paraded daily in morning (7 AM) to detect cows and heifers exhibiting heat and also to confirm the heats detected by Estrone reading. Low Estrone reading and detection of heat by teaser bull were correlated. After confirmation of oestrus the cows / heifers were inseminated with frozen semen of Jersey bull. Following insemination the Estrone reading were continued daily for another 21 days, to note whether inseminated cows / heifers were conceived. The cows and heifers not returning to oestrus within 21 days after insemination were considered as pregnant. However final confirmation of their pregnancies was done on 60th day of insemination by doing per-rectal palpation.

Stastistical analysis of data was carried out according to the method described by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Out of 12 cows, 3 cows exhibited oestrus and out of 6 heifers, 3 heifers exhibited oestrus. These were inseminated with frozen semen of Jersey bull. Out of these 3 cows and 1 heifer conceived. Thus the conception rate of cows / heifers with first insemination was 66.67 percent, as 4 out of 6 cows / heifers inseminated conceived.

The mean Estrone reading of cows / heifers taken during different stages of oestrous cycle were 127.85±6.10, 88.33±0.70, 134.916±3.88 and 145.50±2.56 during pro-oestrus, oestrus, met-oestrus and di-oestrus respectively. The mean Estrone reading of cows and heifers at the time of insemination was 86.50±0.76. These findings indicate that during oestrus stage the average Estrone reading was minimum, there after it raised in met-oestrus and maximum in di-oestrus stage. It was further observed that for cows / heifers which conceived following inseminations, the di-oestrus reading never declined, whereas in those cows / heifers which were not pregnant the dioestrus reading showed sharp decline on 18th day of insemination, indicative of next pro-oestrus.

A positive correlation between low Estrone reading and cows / heifers exhibiting heat was observed. These results are in agreement with those reported by Cerne (1968), Metzger *et al.,* (1972) and Carter and Dubty (1980), who reported that vaginal electrical resistance was lower during oestrus and it was on higher levels during other stages of oestrous cycle in cattle. Thus it can be concluded that the Estrone, electronic vaginal probe may prove benificial in detecting cows / heifers in heat and also may prove beneficial in monitering the optimum time of insemivation.

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Invitro Penetration of Bovine Cervical Mucus by Frozen Bull Spermatozoa

R. ANIL KUMAR AND T.G. DEVANATHAN

Department of Gynaecology and Obstetrics, Madras Veterinary College, Madras-7

ABSTRACT

The variation caused by different cervical mucus samples and different bulls in their sperm penetration capacity invitro was studied. The sperm penetration speed (SPS) for the different bulls varied between 62.64±2.65 μ/Second and 48.61±1.97 μ/Sec. The SPS for different cows varied between 69.72±1.84 μ/Sec and 43.75±0.79 μ/Sec. The sperm penetration speed was significantly different between the bulls (P<0.01) and between the different mucus samples (P<0.01) studied.

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In addition to the conventional criteria of determining sperm motility, density and morphology, the nature of interaction between the sperm and cervical mucus is considered to be an important factor in any investigation of sperm function. One of the method used to evaluate such interaction is invitro penetration of bovine cervical mucus by the spermatozoa. But, such poretration is limited by the mucus collected from different cows (Kummerfold *et al.,* 1981) and the variation in their physiochemical properties (Pattabiraman *et al.,* 1967 a). Hence the present investigation is envisaged to find the variation caused by the different mucus samples for different bulls sperm penetration capacity.

MATERIALS AND METHODS

Frozen semon samples from 6 Jersey bulls processed in 0.5 ml French medium straw were utilised for the study. Cervical mucus was collected from estrous cows using a plastic insemination sheath as per Dabas and Maurya (1988) and evaluated for sperm receptivity (Pattabiraman *et al.,* 1967 b) After evaluation the mucus samples found suitable were loaded into non heparinised homatocrit capillary tubes using a loading manifold. Chinaclay was used to plug the capillary tube and the tubes were allowed to stand for 5 minutes, if mucus contracts during this period the mucus column was adjusted by plugging more clay. The capillary tubes were then suspended vertically in a sperm reservoir which contains, thawed semen sample and the whole setup was incubated at 37°C for 10 minutes. The capillary tubes were removed from the reservoir and the tubes were their placed on graduated glass slide kept on the microscopic stage maintained at 37°C. Spermatozoa swimming in the mucus were observed at a magnification of 400X under the phase contrast microscope.

The distance the spermatozoa travelled in a fixed time (10 minutes) was measured. The average sperm progression speed (SPS) through the mucus column was calculated in micron/Second (Gaddum Rosse *et al.,* 1980). Differences among bulls and individual mucus samples were tested by factorial experiment in randomised block design.

RESULTS AND DISCUSSION

The mean SPS of six bulls tested against 6 cervical mucus sample are presented in table. The mean SPS for the six mucus samples varied from 43.75±0.79 to 69.72±1.84 μ/Sec. Two way analysis of variance showed that SPS in cervical mucus was significantly different between the individual mucus samples (P<0.01). The cervical mucus samples were collected from different mucus donors by careful observation

* Part of the MVSC thesis submitted by the first author to the Tamil Nadu Veterinary and Animal Sciences University.

of the cows for standing estrum (Physical Signs, Contraction of uterus on rectal palpation and presence of a large follicle in the ovary) But the exact begining of the estrum was not known. Thus the variation in the collection time of the mucus (Pattabiraman *et al.,* 1967 b) must have influenced the cervical mucus composition there by affecting the SPS.

The SPS for 6 bulls varied between 62.64±2.65 and 48.61±1.97 μ/Sec. Two way analysis of variance showed a significant variation among different bulls. The variation in the spermatozoal characteristics such as motility, abnormality and acrosome integrity may have contributed for the difference in SPS among the bulls studied. This was in accordance to Bals Pratsch *et al.,* (1988) who found that the seminal parameters allowed a correct prediction of the penetration test (Sperm concentration, morphology and motility) in 54% of cases. The interaction between the Bulls and cervical mucus samples were also significant.

It was concluded that individual bulls vary in their SPS in cervical mucus and individual mucus samples vary in their sperm progression speed.

Table 1. Overall mean sperm progession speed in Bovine cervical mucus

Cervical mucus		Bulls	
No.	μ/Sec	No	μ/Sec
CM1	50.97±1.62	B1	62.64±2.65
CM2	45.83±0.86	B2	54.31±1.82
CM3	43.75±0.79	B3	55.76±2.15
CM4	69.72±0.76	B4	53.26±2.37
CM5	56.87±0.76	B5	61.11±2.68
CM6	68.54±1.57	B6	48.61±1.97

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Testosterone Profile during Estrous Cycle in Cross-Bred Cows

I.J. REDDY, V.P. VARSHNEY, P.C. SANWAL AND J.K. PANDE

Division of Animal Physiology and Climatology, Indian Veterinary Research Institute
Izatnagar, 243 122, U.P. INDIA.

ABSTRACT

This investigation was undertaken to study the circulating androgen profile during estrous cycle in crossbred cows. The plasma testosterone concentration was found lowest (32.19±1.8 pg / ml) on the day of estrus. The levels fluctuated within a range of 49-56 pg / ml upto day 7 of the estrous cycle. Then there was a significant ($P<0.05$) gradual rise on days 8th, 9th and 10th of the estrous cycle. Thereafter, the levels stood low and fluctuated between 53.5 and 65 pg / ml from day 10th to day 16th of the cycle. This was followed by a rise to 60-80 pg / ml on days 19th, 20th, and 21st of the cycle. Higher mean testosterone levels were observed during the follicular and luteal phases. Significantly lower levels were observed during the rest of the cycle. The absolute testosterone levels varied however, amongst the individual animals.

——x——x——x——

The ovary of many mammals is able to secrete androgens (Baird, 1978). Studies on hormone-immuno neutralization (Hillier, *et al.*, 1974) revealed that, the excess level of androgens during estrus suppress the preovulatory LH surge and lead to partial ovulation and anovulation. However, non availability of information on the concentration of testosterone during different phases of estrous cycle, it is difficult to indicate the physiological role of ovarian androgens in the the regulation of gonadotrophin secretion and estrus behaviour. Present study, therefore, attempts at documenting the circulatory testosterone profile in crossbred cows during different phases of estrous cycle.

MATERIALS AND METHODS

Ten crossbred cows of age 8-10 years were involved in this study. The estrus was detected using a vasectomized bull. Jugular venous blood samples were collected daily from all animals throughout the estruous cycle. Plasma was separated out by centrifugation of blood in cold and kept at -20°c untill the analysis. Testosterone was assayed by standard radio-immuno assay (RIA) technique. The results were statistically anlysed as per Snedecor and Cochran (1961)

RESULTS AND DISCUSSION

The mean values of plasma testosterone during different days of estrous cycle are presented in Table 1. The profile of testosterone showed significant variations ($P<0.05$) between days and different phases of estorus cycle. Testosterone records significantly ($P<0.05$) lowest value (32.9±1.89 pg / ml) on the day of estrus, afterwards it gradually increased and fluctuated till day 9, followed by slow decline and another increase thereafter. The values were significantly ($P<0.05$) higher during follicular and luteal phases of the estorus cycle. Thus the trend was similar to observations made earlier in buffaloes (Singh and Madan., 1987) and in ewes (Stankov and Kanchev., 1984). The lowest mean level on the day of estrus, apparently indicate the maximum conversion of testosterone to estradiol. This may have a complimentary effect on estradiol level which is considered to be highest on the day of estrus. However, Peterson *et al.*, (1978) in cows reported higher levels on the day of estrus. Such higher level in this study was observed 1 day before the estrus. Fluctuations in the levels of testosterone during estrous cycle in our study, indicates the pulsatile nature of testosterone secretion (Arora and Pande 1982). Significantly ($P<0.05$) higher values during the luteal phase suggested that corpus luteum and ovarian follicles are able to synthesize

testosterone in vivo (Shemedh *et al.,* 1975). These observations lead to postulate that corpus luteum may be the source of higher testosterone values during the luteal phase. Higher mean levels during luteal and follicular phase of the estrous cycle appear to be associated with higher estrogen fluctuations due to follicular maturation. Mid cycle wave of follicular development in bovines (Rajakoski. 1960) associated with short phase estradiol-17 B elevation and low magnitude LH peak (Madan and Johnson., 1973) could also be correlated to higgher plasma testosterone values. Conclusively, however, this study provides the evidence of relationship of the circulatory testosterone to the luteal regression and it's possible role in regulating the follicular maturation.

Acknowledgement: The author is highly thankful to the Director IVRI, for providing necessary facilities to carryout the present study.

Table 1. Blood plasma testosterone (pg / ml) concentration during estrous cycle in crossbred cows:

Estrous cycle Days	**Mean±S.E.**	**Estrous Cycle** Days	Mean±S.E.
0	32.90±1.89	12	51.44±3.00
1	49.20±2.80	13	61.70±3.00*
2	43.33±2.50	14	59.70±2.78*
3	50.40±8.58	15	63.90±1.98*
4	51.11±1.95	16	65.90±2.26*
5	56.80±4.30	17	74.11±3.72*
6	55.00±3.48	18	82.11±2.83*
7	54.70±3.08	19	68.70±3.00*
8	60.70±3.60*	20	66.50±3.31*
9	80.80±2.23*	21	80.28±2.11*
10	64.80±3.94*	22	81.16±2.30*
11	59.30±5.12*	0	36.11±2.30

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Effect of Heat Stress and Gonadotropic Hormone Treatment on Follicular Atresia

D. ANTOINE[1] AND S.R. PATTABIRAMAN.[2]

Department of Obstetrics and Gynaecology, Madras Veterinary College,
Tamil Nadu Veterinary and Animal Science University, Madras-7.

ABSTRACT

Healthy adult albino female rats were subjected to heat stress at 40.0±0.5°C daily for five days. A group of heat stressed rats were given FSH and LH hormones. Another group received hormones alone. Based of the histology, the atretic follicles were classified into four types (mild to severe). Serial sections of the ovary were used to quantify the changes in the follicular atresia. Heat stress increased the incidence of the atretic follicles even on the fifth day after the heat stress. The high incidence perisisted even upto 15th day post heat stress. The incidence of severe type D atretic follicle, were seen in heat stressed rats. In hormone treated heat stressed rats the incidence of atretic follicle was significantly less than heat stressed rats especially on 10th and 15th post heat stressed days. The incidence of severe type D atresia was also significantly less during the post treatment period.

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Heat stress was observed to significatly affect folliculogenesis (Antoine and Pattabiraman, 1994). It was also recorded that Gonadotropic hormone treatment to heat stressed rats counteract the adverse effect of heat stress. Earlier workers reported that the superovulatory response of Gonadotropin (PMSG) was mainly by rescuing antral follicles from atresia (Braw and Tsafriri, 1980b, and Monniau *et al.,* 1984). The present investigation was taken up to study the effect of heat stress alone and in combination with Gonadotropic hormones on the process of follicular atresia.

MATERIALS AND METHODS

Healthy adult albino female rats aged 120±5 days were utilised for this study. Utilising BOD incubator with humidity control rats were heat sressed at 40.0±0.5°C and RH of 70 per cent for two hours daily for five days. Serum Gonadotropin FSH (Biochem Pharmaceuticals) 10 IU was administered intraperitoneally on day one and Luteinizing hormone (Intervet International) 10 IU was given subcutaneously on day 3 of heat stress period.

Fifty seven rats were utilised for the study. They were divided into Heat stress group (n=16), Hormone treatment group (n=16), Heat stress and hormone treated group (n=16) and Untreated control group (n=9). Four rats in treatment group were sacrificed on 5, 10, 15 and 25 days after the beginning of heat stress. Te untreated control rats were sacrificed separately in batches of three. The ovaries were collected and processed for histological examination (Humason, 1979). Serial thin sections of the ovary were prepared and were analysed.

Based on the description of atretic follicles at different stages reported by Osman (1985) and based on the histological characteristics like pyknotic nuclei in the granulosa cells, cellular debris and infiltration of macrophages in the antrum, thinning of the granulosa cell layer and deformed and vaculoated granulosa cells the follicular atresia in the present study was classified into four types in the increasing order of severity from mild (Type A) to severe (Type D). Number of different types of atretic follicles were counted

1. Asst. Professor, Rajiv Gandhi College of Veterinary and Animal Sciences, Pondichery.
2. Professor and Head, Dept. of Clinics, MVC, Madras-7.

and the percentage of incidence of each type of atretic follicle for the different treatment group was obtained. The data was statistically analysed adopting standard procedures (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

The percentage of incidence of atretic follicle (Fig.1) was 40.0 in control untreated rats. In heat stressed rats the incidence of atretic follicles significantly increased to 74.2 per cent by day five. It remained at 77.4 and 72.8 per cent on day 10 and on day 15 respectively. On day 25 it declined to 54.5 per cent. In hormone treated rats there was no change on day 5 but slight increase in the incidence of atretic follicle to 50.8 was noticed on day 10 which declined to normal level by day 25. When heat stress and hormone treatment were given together the incidence of atretic follicles rose to 68.0 per cent on day 5 but unlike in heat stress declined to 56.8 percent even on day 15 post treatment.

The incidence of different types of atretic follicle in control and treated rats is summerised in table. In control untreated rats mild Type A form of atresia was 60.0±4.2 per cent while the severe type D form was absent. On the contrary in heat stressed rats even on day 5 type D atresia was noticed in 20.2±6.2 per cent of the follicles. Even on day 15 the incidence was high (29.6±7.4 percent). on day 25, 5.6±4.8 per cent of the follicle continued to show severe type of atresia. In hormone treated rats there was slight increase in the incidence of type C and type D on day 10 and 15 but by day 25 the incidence was within normal level. In heat stressed and hormone treated rats there was increase in the incidence of type C and type D atretic forms similar to heat stressed rats but by day 25 the severe type atresia was absent and type C was also only 17.5±6.1 per cent in contrast to 31.3±4.5 per cent of heat stressed rats.

In normal untreated control rats 40.0 per cent of the antral follicles showed atresia. This incidence was within the normal range since Osman (1985) also recorded 35.0 per cent of atresia. The significant increase in the incidence of atretic follicles even on day 5 post heat stress indicate accelerated process of atresia of mature follicles. Further infiltration of large number of macrophages into the antrum of the atretic follicle (Type C and D) was noticed in heat stressed rats. Braw and Tsafriri (1980a) and Osman (1985) reported that infiltration of macrophages as the characteristic feature of the atretic follicle. Gondos (1982) also confirmed such finding based on ultrastructure features.

Earlier reports state that only antral follicles undergo atresia (Butcher and Keller, 1984 and Osman, 1985). The high incidence of atretic follicle on day 10 and 15 post heat stress indicate the possibility that even pre-antral follicles undergo subtle subcellular damage so that they fail to develope to normal antral follicle.

In heat stress-hormone treated group on day 5 the low incidence of atretic follicle could be the effect of the PMSG in reversing the process of atresia. Monniaux *et al.*, (1984) and Moor *et al.*, (1984) observed that superovulation was made possible by the effect of PMSG in reversing the process of atresia. Further the number of atretic follicles and the incidence of severe from of atresia (Type D) were significantly less than in heat stressed rats. This could be due to the effect of gonadotropic hormone to counteract the atresia. A similar anti atretic effect of PMSG on the large mature follicles has been reported by Peters *et al.*, (1975) Peters (1979) and Hay *et al.*, (1979).

Figure

Incidence of Atretic Follicles During Post Heat Stress And / Or Hormone Treatment

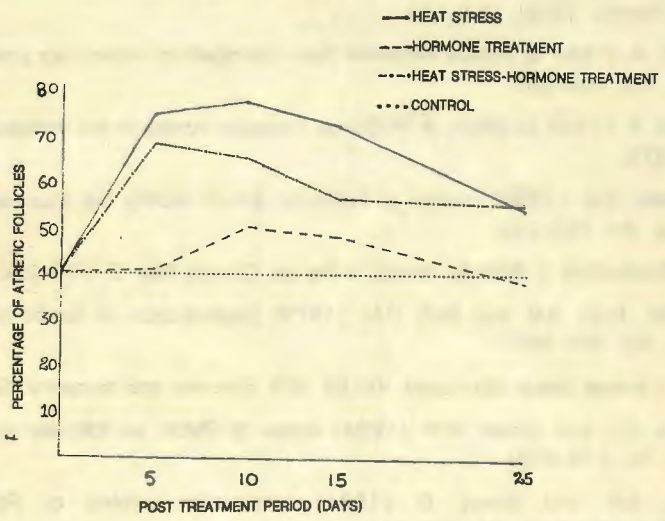

Table 1. Effect of Heat and/or Hormone Treatment on the Incidence of different Types of Atretic Follicle

Nature of treatment and period in days	Percentage of atretic follicle			
	Type-A	Type-B	Type-C	Type-D
Control	60.0±4.2	30.0±6.5	10.0±6.8	—
Heat stress				
5	30.2±7.0*	33.3±7.3	16.3±5.7	20.2±6.2
10	10.4±4.9*	46.5±8.0**	13.5±5.5	29.6±7.4
15	18.6±4.5**	32.0±5.4	25.3±5.0**	24.1±4.9
25	36.5±4.6**	26.6±4.3	31.3±4.5**	5.6±4.8
Hormone treatment				
5	72.2±6.8	20.7±9.8	7.3±10.7	—
10	68.9±8.7	17.8±7.2*	10.4±5.8	2.9±2.5
15	64.5±8.8	13.8±6.4**	20.7±7.5*	1.0±1.6
25	57.0±6.1	28.0±9.1	15.0±7.2	—
Heat stress and Hormone treatment				
5	47.5±7.0*	31.4±6.5	15.8±5.2	5.3±1.0
10	37.5±7.7**	30.0±7.2	25.0±6.9**	7.5±2.9
15	30.0±6.5**	36.0±6.8	30.0±6.5**	4.0±1.9
25	52.0±7.1	30.5±6.1	17.5±6.1*	—

Values marked (**) or (*) are significantly different from control value of corresponding type.

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Role of Prefreeze Cooling / Holding Times, Dilutors and Thaw rates in Improving Freezability and Post-thaw Survival of Bovine Spermatozoa*

A.J. DHAMI[1], K.L. SAHNI AND GREESH MOHAN

Division of Animal Reproduction, Indian Veterinary Research Institute, Izatnagar 243122, Uttar Pradesh

ABSTRACT

Split-samples of 18 semen ejaculates of 3 Friesian bulls were frozen in Tris and milk-based diluents following various prefreeze cooling (10°/30°C; 1 & 2 h) and equilibration (0 & 2 h) treatments and were thawed at 4°, 40° and 60°C for 300, 60 and 15 sec, respectively. The prefreeze motility was reduced significantly following both 1 and 2h of cooling directly from 10°C as compared with 30°C. However, the post-thaw recovery (PTR) and incubation aging survival of sperm was significantly higher with 2 h of cooling from both 10°C and 30°C than with 1 h of cooling. Sperm post thaw motility was significantly better after 2 h of equitibration over oh with tris than milk and with faster thaw rates. An acceptable level of sperm forward motility was maintained till 45 min of post-thaw incubation and upto 36 h of post-thaw aging. The fertlity rates of frozen semen had significant positive correlations with sperm motility (r=0.26 to 0.50), and negative correlations with GOT leakage (r= -0.61 & -0.65) pre-and post-freezing.

——x——x——x——

The role of different variables in deep freezing of bovine semen has yet not been well eatablished except that frozen-thawed sperm survive for a much shorter time than the unfrozen sperm (Venguest *et al.,* 1984). This paper reports the effects of variables, particularly thaw rates and post-thaw thermal strees, on incubation (37°C)/aging (5°C) survival of bull spermatozoa and its correlations with GOT leakage and fertility rates of frozen semen.

MATERIALS AND METHODS

Semen ejaculates (18) obtained at weekly interval from 3 Friesian bulls of IVRI were split-diluted at 32°C in Tris-fructose-yolk-glycerol and Cow's whole milk-yolk-glycerol diluents, keeping 55-60 million sperm/ml. French medium straws (56/diluent) of 4 different colours were filled with each sample. One forth straws (14 Tris + 14 milk) each were placed in 4 bread boxes containing measured quantity of water either at 10° or 30°C and cooled to 5°C over 1 and 2 h each in a refrigerator as described earlier (Dhami and Sahni, 1993). On cooling to 5°C, half the straws (7+7) from each box were frozen in liquid nitrogen vapours (O h equilibration) and the remaining straws were equilibrated for 2 h. The straws were thawed in water-baths at 4°, 40° and 60°C for 300, 60 and 15 sec, respectively. One straw thawed at each rate was soon transferred to an incubator (37°C) and the another one to a refrigerator (5°C) for post-thaw thermal stress. Sperm forward motility at prefreezing and after 0, 30 and 60 min of post-thaw incubation and 6, 24 and 48 h of aging was assessed under a phase contrast microscope fitted with a biotherm. The data were analysed using a 5 factor factorial RBD and were correlated with GOT leakage and fertility rates already published (Dhami and Sahni, 1993).

RESULTS AND DISCUSSION

The overall mean initial motility of 82.7% was found to decline significantly at pre-and post freezing to 66.8 and 39.5%. This further declined to around 30% after 30 min of

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Current address:

1. Associate Professor, Gynaecology and Obstetrics, Gujarat Veterinary College, GAU, Anand-388001.

incubation or 24 h of aging, and to 20% after 1 h and 48 h of storage, respectively (Table 1). These findings coincided well with the earlier reports on freezability/post-thaw incubation (Tuli *et al.,* 1985; Chinnaiya and Balakrishnan, 1988) and aging survival (Sahni and Mohan, 1988; Mathur *et al.,* 1990) of bovine semen. Statistically, the influence of bulls, diluents, cooling rates, equilibration periods and thaw rates was highly significant ($P<0.01$) for post thaw incubation/aging motility at all intervals. Amongst the 2-way interactions, only the bull x equilibration and bull x thawing interactions were significant. This suggested that sperm from individual bull behaved differently at different equilibration and thawing temperatures. Tuli *et al.* (1985) and Dhami and Sahni (1993) also reported similar interactions for bull and buffalo semen.

The deterioration in sperm motility at prefreezing was significantly ($P<0.05$) greater after faster cooling of straws in 1 h directly from 10°C (60.4%) as compared to 1 h of cooling from 30°C (67.9%) or 2 h of cooling from 10°C (66.6%), and the values of all 3 rates were significantly lower than that of slow cooling over 2 h from 30°C to 5°C (73.4%). These initial temperatures had, however, no effect on post thaw motility and incubation and aging survival of sperm. The 2h of cooling was significantly ($P<0.01$) superior over 1 h for both the temperatures. Not only the slower cooling from 30° to 5°C, but also the longer equilibration of at least 2 h at 5°C, compared with 0 h, significantly enhanced the freezability, post-thaw incubation/aging survival and fertility rates of bull spermatozoa (Table 1, Fig. 1). Further, the pre-and post-freeze motility, GOT leakage and fertility rates did not vary between Tris and milk diluents, but the post-thaw incubation and aging survival of sperm was significantly lower in milk than in Tris (Table 1). This might be due to loss of buffering capacity and increased microbial growth on storage in former biological medium.

Our findings with regard to the need of gradual slow cooling of bovine semen compared well with the earlier observations of many workers (Wiggin and Almquist, 1975; Mathur *et al.,* 1990). This was also supported by our findings of significantly lower GOT leakage and higher fertility rates of frozen semen produced after slow cooling form 30°C than from 10°C and also after 2 h of equilibration than after 0 h (Fig.1). The subjective index of post-thaw incubation/aging survival of sperm did not vary between 10°C and 30°C in our study after 2 h of cooling, yet the fertility rate was significantly higher and GOT leakage lower with 30°C (Fig.1). This indicated that the drastic cooling of bovine semen from 32°C to 10°C water was detrimental to sperm cell membrane integrity and fertilizability, and hence must be avoided. Our results on post thaw incubation and/or aging survival of bovine sperm in Tris/milk diluents coincided well with the findings of Chinnaiya and Balkrishnan (1988) and Mathur *et al.* (1990), and suggested that there is no harm in using whole milk as a cheaper and readily available substitute to costlier Tris.

Three thaw rates used also varied significantly ($P<0.01$) in respect of post-thaw recovery and incubation/aging survival of sperm. The values were in favour of higher thawing temperatures of 60°C or 40°C. Samples thawed at 60°C/15 sec showed significantly better post-thaw recovery and sustenance of sperm motility on incubation/aging as compared to those thawed at 4°C for 5 min (Table 1). Similar observations were also made by others (Tuli *et al.,* 1985; Sahni and Mohan, 1988) Robbins *et al.,* (1976) advocated a thaw rate of 50°C/15 sec or 65°C/7.5 sec for bull semen. Chinniya and Balakrishnanan (1988) found a gradual rise in conception rates with the use of semen having increasing post-thaw survival at 38°C. Though we have not conducted any fertility trial with different thaw rates, we suggest a higher thaw rate of 60°C/15 sec in view of above reports and on the basis, better the post-thaw motility greater the fertility, as we observed with various cooling and equilibration treatments (Fig.1).

There were significant positive interrelationships between sperm forward motility at various incubation/aging intervals and fertility rates ($r=0.26$ to 0.99). The GOT leakage pre-and post-freezing had positive correlations with post-thaw incubation/aging motility ($r=0.26$ to 0.38) and negative correlations with fertility rates

(r= -0.61, -0.65) (Table 2). Pace *et al.,* (1981) also found positive correlations between sperm motility estimates immediate after thawing or after 1-2 h of post-thaw incubation (37°C) and fertility rates and/or proacrosin content. Uwland (1984) observed significant positive correlation of 0.53 between post-thaw aging (5°C) survival after 24 h and nonreturn rates. Our findings thus support these reports and confirm that frozen semen having bettern post-thaw recovery and survival should only be used in artificial breeding programme within 30 min of thawing for acheiving higher conception rates.

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Table 1. Mean post-thaw (PT) recovery and incubation (37°C)/aging (5°C) survival of bull spermatozoa (%) as influenced by different treatments.

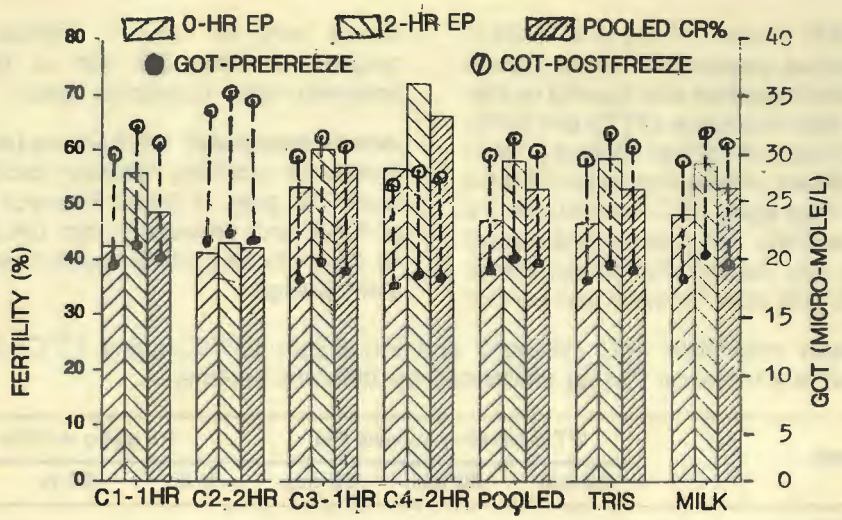
Treatment		PT incubation survival (%)			PT aging motility (%)		
		00 min	30 min	60 min	6 hr	24 hr	48 hr
Cooling rate/time:							
10° to 5°C,	1 hr	35.5±0.7	27.1±0.8	15.4±0.8	30.4±0.8	24.1±0.9	17.0±0.8
	2 hr	43.9±0.8	34.6±0.9	24.2±0.9	38.6±0.7	31.9±0.8	23.6±0.8
30° to 5°C,	1 hr	34.2±0.8	26.7±0.8	15.6±0.7	30.3±0.8	24.4±0.8	16.5±0.8
	2 hr	44.4±0.7	34.8±0.8	23.6±0.8	38.6±0.8	32.5±0.9	23.7±0.9
Equilibration period:							
	0 hr	35.3±0.6	26.5±0.6	15.7±0.5	30.3±0.6	24.1±0.6	16.9±0.5
	2 hr	43.5±0.5	35.1±0.6	23.6±0.6	38.7±0.5	32.5±0.5	23.5±0.6
Dilutors:							
	Tris	40.0±0.6	31.4±0.7	20.8±0.6	35.2±0.6	29.3±0.6	20.9±0.6
	Milk	38.1±0.6	30.2±0.6	18.6±0.6	33.7±0.6	27.2±0.6	19.6±0.6
Thaw rates:							
	4°C	33.0±0.6	24.2±0.7	13.6±0.6	28.2±0.6	22.1±0.6	15.0±0.6
	40°C	39.8±0.6	31.4±0.7	20.2±07	34.9±0.7	28.4±0.8	20.3±0.7
	60°C	45.8±0.6	36.7±0.8	25.2±0.7	40.4±0.7	34.2±0.7	25.3±0.7
Overall Pooled:							
		39.5±0.4	30.8±0.5	19.7±0.4	34.5±0.4	28.2±0.5	20.2±0.4

'F' test for all variables was highly significant (P<0.01)

Table 2. Correlation coefficients between motility, GOT leakage and fertility rates of bovine frozen semen.

Characteristic	PTM	PTI-1h	PTA-6h	PTA-48h	GOT-PF	GOT-PT	CRs
prefreeze motility	0.30	0.34*	0.32*	0.32*	-0.59**	-0.42*	0.50**
Post-thaw motility	—	0.99**	0.99**	0.99**	0.29	0.38*	0.26
PTI 1 hr		—	0.99**	0.99**	0.29	0.38*	0.29
PT aging 6hrs			—	—	0.27	0.35*	0.33*
PT aging 48hrs				—	0.26	0.34*	0.31*
GOT prefreeze					—	0.97**	-0.61**
GOT post-thaw						—	-0.65**

* P<0.05; ** P<0.01; PTI = Post-thaw incubation; PTA = Post-thaw aging.



Fertility of frozen bull semen as influenced by various cooling rates (C1, C2 from 10° to 5°C and C3, C4 from 30° to 5°C), equilibration periods (EP, 0-2 hr at 5°C) and dilutors in relation to enzyme GOT leakage pre and post-freezing

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Study on Biochemical Aspects of Male Gonad (Surti Buffalo) At Four Stages of Development

SHERSINGH CHAMPAWAT,[1], M.M. PATHAK[2] and K. JANAKIRAMAN[3]

Reproductive Biology Research Unit and H.F. Project,
Gujarat Agricultural University, Anand Campus, Anand - 388 110

ABSTRACT

Farm born Surti males at four developmental stages, six in each, impuberal (one month age), prepuberal (10 months age), puberal (18 months age) and postpuberal (24 months age) were selected for the biochemical study of the testis. Results have indicated that nucleic acids (DNA), phosphatase enzyme (AKP), cholesterol, protein and phosporus have shown significant correlation with the development of gonad. Acid phosphatase (ACP) did not show any significant relation with development and functional aspects of gonads.

——x——x——x——

The biochemical constituents of an organ are involved in many metabolic pathways which are essential for functioning of the organ. In the present study an attempt has been made to know the biochemical constituents of testis at four stages of development in Surti Buffalo.

MATERIALS AND METHODS

Surti calves at four developmental stages, 6 animals in each stage were studied: Stage I (Impuberal) one month age; Stage II (pre puberal) 10 months age; Stage III (puberal) 18 months age; Stage IV (post puberal) 24 months age.

Testis were removed surgically from all these animals and homogenate were prepared for biochemical study (under cold condition). The characters analysed were : Nucleic acid (DNA - RNA - Scheider 1957); enzymes phosphatase (AKP - alkaline phosphatase and ACP - acid phosphatase King and Armstrong 1934); total protein (Lowry *et al.*, (1951), Cholesterol, (Schoenheimer and Sparry 1934) and Phosporus (Fiske and Subbarao 1925).

The data were analysed statistically with the help of HCL (Hindustan Computer Limited) Computer.

RESULTS AND DISCUSSION

Nucleic acids: The difference in the levels of DNA due to stages was statistically significant ($P<0.05$) but not for RNA. The concentration of DNA decreased significantly from stage I (impuberal) to stage III (puberal) and than slightly increased to stage IV (post puberal) while RNA concentration did not show any specific fluctuation except highest level in post pubertal stage (Table 1), Ratio of RNA : DNA was increasing from stage I (0.64) to IV (1.05). Same type of results have been reported by Deshpande and Janakiraman (1985) in their study on histology and histochemistry of Surti buffalo testis.

Higher level of DNA in stage I and II (impuberal and prepuberal) can be explained as due to large nucleus of spermatocyte during late pachytene stage of spermatogenesis in buffalo (Guraya and Bilaspuri 1976). Further decrease of nucleic acids at stage III may be due to increase in tubule diameter and increase in the cell number per tubule, which correlates with progressive disappearance of RNA with relative increase in sperm cell (Mann, 1964).

The correlation study between the biometrical observations and DNA content of the testis was significantly negative ($r=0.46$). Increase in size of testis decreases DNA content. The ratio of DNA / RNA also reduced which probably explains the transformation of spermatocytes to

Present Address:
(1) Assoc. Research Scientist, H.F. Project, IDCP / NDDB Unit, GAU, Anand Campus, Anand.
(2) Research Scientist, R.B.R. Unit, GAU, Anand Campus, Anand.
(3) Major Professor and Director of Research, GAU, Ahmedabad.

spermatozoa and enlargement of tubes (Desjardines *et al.,* (1968).

Phosphatase enzymes: Alkanine phosphatase activity showed some significant variation between the stages of development (Table 1). Same type of results in testicular enzyme have been reported by Deshpande and Janakiraman (1988). Higher activity in animals of stage I may be a reflection of higher metabolic rate. Elevation of testicular AKP in animals of pubertal stage (III), a stage of full fledged spermatogenesis, may be explained as influence of increased testosterone which has positive association (Choudhary and Mukherjee 1977). In support of this, Rollinson(1954) reported that AKP activity in testis was correlated with fibrillar tail formation at pubertal age.

Acid phosphatase did not show any specific correlation with development of testis as well as physiology of testicular function. The fluctuations recorded may reflect only production and utilization during development.

Total Protein: Optimum level of protein is a must for the development of endocrine and sex organs (Herrick, 1977). In the present study, excluding the 2nd group (10 months age), the testicular protein showed declining trend (Table 1), which was statistically significant. These results are comparable with results of Deshpande and Janakiraman (1985). The higher levels of testicular protein in the animals of Group I, may be due to histological status of testis which includes solid seminiferous cords with gonocytes and basal indifferent cells. Further increase in testicular protein at pubertal stage (Stage II) may be due to higher level of mitotic activity in testis.

Correlation between the biometrical observation of testis and testicular protein showed a significant negative correlation (r= -0.61). Same way between serum testosterone also had negative correlation (r = -0.125). These facts are represented by linear and considerable decrease in testicular protein with advancement of age (Stage I to IV Table 1).

Cholesterol: Both total and free cholesterol of testis showed linear decrease from stage I to IV. The differencs were statistically significant (Table 1). These levels and the trend are comparable with the results of Deshpande and Janakiraman (1987). Overall ratio of total and free cholesterol was more than two Ewing *et al.,* (1966), stated that the cells in advanced stage of spermatogenesis are associated with deplition of cholesterol from the testis as it matures. As spermatozoa can catabolize the cholesterol, the reduction in testicular cholesterol in matured male may be due to higher number of spermatozoa and spermatids (Johnson *et al.,* (1970). Correlation study had shown a negative correlation between serum testosterone and testicular cholesterol (r= -0.351).

Phosphorus: The variations observed in the level of testicular phosphorus between the stages were statistically significant. The level was highest in stage II i.e. prepubertal stage and lowest in stage III i.e. pubertal stage. These levels are some what lower than those reported for bull (Mann 1964). As such, there is no work reported on testicular phosphorus in buffaloes. Reduction of phosphorus level in pubertal and post pubertal animals indicates its utilization at initiation of spermatogenesis as phosphorus is known for its role in carbohydrate metabolism (Hackett *et al.,* (1957) which is source of energy.

Overall, the testicular biochemical aspects give their importance and association with development and functional aspects of testis in Surti buffalo males.

Table 1. Overall mean±S.E. for Biochemical characterastics of testis.

Characters	Stages				Signicicancy
	I	II	III	IV	
RNA (mg / g)	3.82±0.41	4.13±0.46	3.31±0.42	4.39±0.39	N.S.
DNA (mg / g)	5.97±0.43	5.87±0.41	3.76±0.26	4.17±0.39	(P<0.05)
AKP (KAU / g)	44.73±4.40	10.70±1.21	23.27±1.81	13.69±1.28	(P<0.05)
ACP (KAU / g)	8.56±0.87	6.83±0.81	8.81±0.78	6.44±0.98	N.S.
Protein (mg / g)	96.43±3.00	102.41±3.34	81.61±5.51	69.72±3.67	(P<0.05)
Phosphorus (mg / g)	3.17±0.35	4.23±0.37	1.29±0.19	2.30±0.37	(P<0.05)
Cholesterol (mg / g) (Total)	11.87±0.60	8.43±0.69	8.82±0.89	6.43±0.63	(P<0.05)
Cholesterol (mg / g) (Free)	4.83±0.56	3.13±0.27	2.96±0.31	2.47±0.25	(P<0.05)

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Antigenecity of Washed-bull Spermatozoa in Model Rabbits and Effect of Cortisone on Immunosuppression

S. BASU, S.K. BONDOPADHYAY, R. ROY CHOUDHURY AND B.B. GHOSH

Department of Animal Gynaecology and Obstetrics
College of Veterinary and Animal Sciences
Mohanpur, Nadia, West Bengal 741 252r

ABSTRACT

Twelve mature albino female rabbits were inoculated with washed bull spermatozoa weekly for 5 consecutive weeks and serum samples collected a week after each inoculation were found to contain antispermatozoal isoantibodies as recorded by counter immunoelectrophoresis (CIEP) and Tube-slide agglutination test (TSAT), whereas control sera gave negative results. A steady increase in the titres of serum isoantibodies were observed in TSAT as the number of inoculations increased and there existed highly significant differences ($P<0.01$) between the titres of immunized does and the controls at different stages of sera collections. Treatment of the hyperimmunized does with Cortisones indicated immunosuppression.

——x——x——x——

In the present investigation anti-washed-bull spermatozoal isoantibody was raised in rabbits and a study on the reversibility of the induced hyperimmune condition was carried out using cortisones which might be a very important direction towards the future study of immunological / unexplained infertility and immunocontraception.

MATERIALS AND METHODS

The antigenic material was prepared by washing the bull semen (X HF) thrice with sterile Phosphate-buffered saline (PBS; pH 7.2) and was resuspended in PBS so as to contain 60 x 10^6 sperm cells / ml. Twelve sexually mature female albino rabbits *(Oryctolagus cuniculus)* were inoculated individually following the method of Sudarsanan *et al.*, (1986) for five consecutive weeks, whereas six control mature female rabbits were given sterile normal saline injections only. Serum samples were collected a week after every inoculation / injection from the heart blood of each of the eighteen rabbits and were stored at —20°C temperature.

The serum samples were then tested for the appearance of any isoantibodies corresponding to the washed bull sperm antigens by CIEP and the progressive development of isoantibody titres (if any) with subsequent inoculations were measured by TSAT following the method of Franklin and Dukes (1964). The methodology of CIEP as adopted was described by Rose *et al.*, (1976) with some modifications as suggested by Basu (1993).

Forty days after the last inoculations / injections, serum samples of all the eighteen rabbits were tested by CIEP and TSAT in order to assess the presence and any depletion in the titre of isoantibodies respectively. Then half of the test rabbits were injected for 4 consecutive days with 1 mg / kg b. wt. / day Crystaline suspension of Prednisolone acetate (Hostacortin-H^R, Hoechst India Ltd.) and the rest half similarly received 0.5 mg / kg b. wt. / day Dexamethasone Sodium Phosphate (Decadron^R, Merind India Ltd.). After 3 days serum samples were harvested and were again subjected to CIEP and TSAT to ascertain the effect of cortisones on antibody titres. The calculations of geometric mean of serum antibody titres and the 't'-test of significance was performed according to Snedecor and Cochran (1971).

RESULTS AND DISCUSSION

The results of both CIEP and TSAT clearly indicated that isoantibodies against washed bull spermatozoa appeared in all test serum samples after the very first inoculation. In CIEP, it was indicated by the formation of precipitation bands in the agar gel. Similar bands were also found with all other test serum samples collected after subsequent inoculations, but none was formed with the control sera (Table 1). In TSAT, the positive agglutination was evidenced by sedimentation of agglutinated masses clearing the suspended medium of the tubes and also microscopically where orderly clumps of head-to-head type sperm cells were formed with their free tails directed towards the periphery. All the control sera gave negative results. It was revealed that there were sharp increase in the levels of circulating isoantibody of any individual rabbit as the number of inoculations increased and there existed highly significant ($P<0.01$) differences in the isoantibody titres between the immunized and control females at all 5 stages of serum collections (Table 1).

Both CIEP and TSAT indicated that washed bull spermatozoa was very strongly antigenic in rabbits as was also found by Hunter and Hafs (1964) who reported 7 sperm specific antigens of protein and glycoprotein in nature with five-times washed bull spermatozoa. The steady increase of serum isoantibody level as observed was very much comparable with the findings of Sudarsanan *et al.,* (1986) with washed buffalo spermatozoa as antigen. The higher antibody titre found in his study might be due to the difference in the antigenic constituents of the spermatozoa of the two species. The specific agglutination occurred in TSAT was considered due to antibodies (immunoglobulins) of either IgG or IgM or both classes as evidenced by fractionation of active sera (Rose *et al.,* 1976) and by using ELISA technique (Lander *et al.,* 1990).

Test serum samples collected 40 days after the last inoculation showed still positive reactions in CIEP and little decreased values of isoantibody titres in TSAT (Table 1) probably because of the time lapses.

CIEP study with the test serum samples collected 3 days after the cortisone treatment indicated that detectable amount of isoantibody was still existed. But results of TSAT showed a sudden and vigorous drop in the titres of serum isoantibodies in the cortisone treated does (Table 1). Both Prednisolone and Dexamethasone gave almost similar results of immunisuppression and the findings were corroborated well with the similar observations made by Curtis *et al.,* (1982) in monkeys and Alexander *et al.,* (1983) in human. Cortisone induced immunisuppression was mechanised possibly due to inhibition in the synthesis of B and T lymphocytes and also macrophages, inhibition in the differentiation of B lymphocytes to antibody producing plasma cells, lysis of activated lymphocytes, inhibition in the production of interleukin-2 and other lysosomal enzymes and mediators, inhibition of antibody synthesis and increased catabolism of IgG (Hanson and Wigzell, 1985).

Table 1. Results of CIEP and TSAT in immunized and control rabbits at different stages of sera collections.

Stages of collections	Immunized group(12)		Control group (6)		't' values
	CIEP	TSAT	CIEP	TSAT	
After first week	+	19.02±0.075	—	0.±0	11.84**
After second week	+	85.31±0.078	—	0.±0	17.24**
After third week	+	203.10±0.077	—	0.±0	20.79**
After fourth week	+	644.50±0.125	—	0±0	15.69**
After fifth week	+	1289.00±0.125	—	0±0	17.38**
40 days after fifth inoculation	+	966.00±0.167	—	0±0	
3 days after cortisone treatment	+	14.92±0.054	—	0±0	

** (P<0.01) (Highly significant)
Figures in parentheses indicate the number of observations

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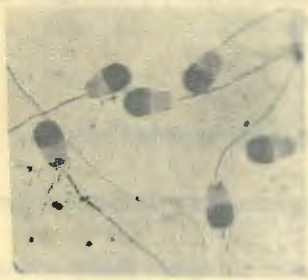
A Simplified Staining Technique for Evaluation of Acrosomal Status of Sperm Cells

P.V. SARMA

Southern Regional Station
National Dairy Research Institute, Bangalore - 560 030

One among semen evaluation tests is assay of acrosmal status by vital dye staining of spermatozoa for its integrity and physical defects since both these parameters are related to fertility. Conventionally, Giemsa staining technique (Hancock, 1952) is followed for staining the spermatozoa in a semen smear which provides a reasonable information on acrosomal integrity and abnormalities in any given semen sample, before and after its cryopreservation. Many times this technique fails to yield satisfactory picture of acrosomes due to development of hazy background especially when the semen sample is mixed with semen extenders containing egg yolk and / or glycerol. Addition of egg yolk beyond certain level was found to interfere with reaction of dye solution (Benjamin *et al.,* (1990) both in chilled and frozen semen. As the level of glycerol increases in semen extenders the permeability of live and motile sperms to the staining were also found to increase (Mixner and Saroff, 1954) which may contribute to the poor staining quality commonly encountered during semen evaluation.



A thin smear of semen drop is made on a clean glass slide and air dried. The dried smear is then immersed in a staining jar containing methanol for 15 minutes at room temparature. The slides are then air dried and transfered to another jar containing staining solution consisting of Stock Giemsa stain 6 cc, 0.2M phosphate buffer, pH 7.4, 4 cc., and carbondioxide free water 90 cc. The stock Giemsa stain is prepared by dissolving one gram of Giemsa powder in 66 cc of methanol and 60 cc of glycerol. After complete dissolution of giemsa powder, the staining solution is filtered and preserved in air tight containers. The slides were left for staining at 37°C for 3-4 hours, and then the slides were removed, washed in tap water and air dried. They were examined microscopically under oil immersion. This simplified technique had yielded very good results with improved clarity and contrast with respect to acrosomal morphology (Fig. 1) in undiluted, extended and frozen-thawed semen. This technique differs from the original method of Hancock (1952) in deletion of pretreatment of semen smears with 5% formaldehyde for 30 mts at 37°C and washing of the smear with water after formaldehyde treatment.

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Effect of Prostaglandin F_2 alpha on Seminal Attributes in Crossbred Bulls

D.B. DIGHE, S.R. CHINCHKAR, V.L. DEOPURKAR, ASHA MANTRI and M.B. MANTRI

Bombay Veterinary College,
Parel, Bombay - 400 012.

Prostaglandins are secreted by almost all the body tissues and most of them act locally at the site of production on a cell-to-cell interaction. It helps in transporting spermatozoa in male genital ducts by centractions of smooth muscles. The effect of different doses of PGF_2 alpha on seminal attributes have been reported by Marshall and Hafs (1979) and Rao *et al.,* (1986).

In the present study, attempts have been made to increase the volume and sperm concentration in two crossbred bulls by injecting two different doses of PGF_2 alpha parenterally.

The experimental period of nine weeks was divided into three equal phases. During all these three phases, semen evaluation was carried out by taking two collections at an interval of 15 minutes per day, twice a week.

Phase I included normal semen evaluation. During Phase II, first ejaculate was collected 30 minutes after the injection of 5 mg PGF_2 alpha whereas in Phase III, first ejaculate was collected 30 minutes after the injection of 10 mg PGF_2 alpha. In all, 12 ejaculates from each bull were collected during each experimental phase to study the seminal attributes.

The average ejaculate volume in Phases I, II and III was 7.81±0.46, 6.87±0.20 and 6.53±0.38 ml respectively. It was observed that there was non-significant decrease in ejaculate volume from Phase I to subsequent Phases which corroborates the findings of Marshall and Hafs (1979) and Hashizume and Niwa (1984).

The average total sperm concentration in Phase I, II and III was 1027.47±82.75, 1069.20±59.94 and 1077.58±89.39 million / ml respectively. There was non-significant increase in total sperm concentration after injecting 5 mg PGF_2 alpha than in Phase I. This is in agreement with the findings of Marshall and Hafs (1979) and Rao *et al.,* (1986).

As the dose of PGF_2 alpha was increased from 5 to 10 mg, the mass activity, initial motility and total sperm concentration were found to be increased with non-significant differences. This is in close agreement with the findings of Ibrahim (1988) in buffaloes.

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Relationship between Scrotal Circumference and Seminal Characteristics in Crossbred Bulls.

K. BABU RAO AND V.V.V.S. PRAKASA RAO

Southern Regional Station
Cattle Project, L.R.S., Lam farm, Guntur-34

The scrotal circumference is a simple repeatable method of measuring testicular size, which reflects on spermetozoa producing ability of the bull, and it has been confirmed in exotic bulls (Kupperschmed *et al.,* 1975 Almquist *et al.,* 1976 and Jakubee, 1984). The present study is aimed to know the relationship between scrotal circumference and seminal parameters in crossbred bulls.

Three hundred and six ejaculates were collected at weekly intervals from 30 crossbred bulls aged between 30 to 105 months. These animals were maintained under identical condition at Cattle Project, Lam Farm, Guntur. Evaluation of semen was carried out as per Rao (1971) and scrotal circumference was measured as described by Hahn *et al.,* (1969). Based on scrotal circumference the bulls were divided into 3 groups i.e. group I <30 cm, Group II 30-35 cm, and Group III > 35 cm to study the relationship between the scrotal circumference and seminal characteristics. The data was subjected to statistical analysis as per Snedecor and Cocharan (1967).

The statistical analysis of data revealed a significant ($P<0.01$) positive correlation between the scrotal circumference and ejaculate volume ($r=0.60$) mass activity ($r=0.72$), sperm motility ($r=0.41$) concentration of sperm per ml. ($r=0.78$) and concentration of sperm per ejaculate ($r=0.72$). Similar observations were made by Coulter and Foote, (1977) and Mohanty *et al.,* (1988) in exotic bulls. Further a significant ($P<0.01$) negative correlation was observed between scrotal circumference and total sperm abnormalities ($r=-0.60$), and proximal droplets ($r=-0.74$). A similar correlation was reported by Mohanty *et al.,* (1988) in exotic bulls.

The results in present study indicates that the greater testicular circumference might result in good quality of semen, which might be due to high spermatognic activity in larger area.

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Comparative Study of some Haematological Parameters in Regular Breeding, Repeat Breeding & Anoestrus Sahiwal Cows

J.R. KHAN, U.K. MISHRA, O.P. MISHRA

Department of Veterinary Physiology
College of Veterinary Science & A.H. Anjora,
Durg (M.P.) - 491001.

The study of normal haematological values of animal is essential in diagnosis of various Physiological disorders and Pathological conditions. The present study therefore has been undertaken to find out comparative haematological pictures in regular breeding, repeat breeding and anoestrus Sahiwal cows.

The study was conducted on 12 Sahiwal cows of research dairy unit. Out of these animals 4 were repeat breeding cows which did not conceive even after 3 or more insemination. Four were regular breeding cows which conceived with one insemination and four animals were anoestrus which did not show any sign of postpartum oestrus. All these above animals were reared under identical managemental and nutritional conditions. The blood samples from each of these animals were collected for 3 consecutive day. The haematological parameters viz. TEC, TLC, haemoglobin, PCV, MCV, MCH, MCHC (Schalm *et al.,* 1975) were studied. The data were statistically analysed (Snedecor and Cochran, 1967).

The Hb, TEC, TLC, PCV, MCV, MCH, MCHC do not show any significant change in between the regular breeding cows, repeat breeding and anoestrus cows. The values were within normal physiological range.

However, the regular breeding cows shows a slight non-significant increase in Hb, TLC, MCH and MCHC as compared to repeat breeding and anoestrus cows. The present values are in agreement to Pyne and Maitra (1981) in Sahiwal cows and Kumar and Sharma (1991) showed lower haemoglobin concentration in repeat breeders however Awasthi and Kharche (1987) did not find any difference in haemoglobin levels in regular and repeat breeding cows. All the above values in Sahiwal cows are in close approximation with Greatorex (1957) in dairy cows.

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Effect of "Receptal" to improve the fertility in bovines

G.P. ROY, M.H. AKHTAR, A.P. SINGH,
K.M. PRASAD AND R.B. SINGH

Department of Gynaecology, Obstetrics and Artificial Insemination.
Bihar Veterinary College, Patna - 800 014.

Anovular heat and cystic ovary conditions on account of hormonal imbalance, specially of GnRH (Hypothalamic hormones) cause failure of fertilization and subsequent poor conception. Such cases were treated with hormones with variable results (Singh *et al.,* 1985; Sankaralingam and Duraisamy, 1986).

In the present study 98 randomly selected crossbreds comprising of 70 cows and 28 heifers brought to Gynaecology clinic at Bihar Veterinary College, Patna were used. These animals were cyclic and had normal genitalia. The animals were divided into two groups. Group-I had sixty animals (control) did not receive any treatment. Thirty eight animals (30 cows & 8 heifers) were treated with 5ml receptal (Hoechst) through i.m. route at the time of onset of heat (Group II). Animals were inseminated and followed up, 60 days after, to record pregnancy.

The conception rate (CR) in control group was 33.3 percent (20 conceived out of 60). In treatment group 73.6 percent animal conceived, 21 out of 30 cows (70 percent) and 7 out of 8 heifers (87.5 per cent) conceived. The study indicates that Receptal (GnRH) can bring improvement in conception rate among those animals suffering from anovular heat and cystic ovarian degeneration.

It is also supported by Sandhu and Singh (1992), who found an overall CR of 67.2 percent in repeater cows.

In the present study favourable response was more in heifers than in cows. This could be due to the fact that unlike cows heifers suffer only from hormanal disturbance and they were not under lactational stress.

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Studies on Efficacy of Receptal (GnRH analogue) to Induce Oestrus in Anoestrus Cows

S.D. SONWANE, D.R. PARGAONKAR, S.A. BAKSHI, R.M. NAVTAKE AND N.V. THAKRE

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

In the present study, efficacy of gonadotrophin releasing hormone analogue (Receptal) to induce oestrus in anoestrus cows was studied.

Twenty four Red Kandhari cows from Red Kandhari cattle unit of this college were selected to study the efficacy of Inj Receptal (GnRH analogue) treatment to induce oestrus. The cows were divided into three groups eight cows in each group. Group I animals were treated with 5 ml Receptal as i.m.injection. Group II animals were given 2.5 ml Receptal by vulval submucosa route. Group III animals remained as untreated control. Cows in all the three groups were daily examined per rectally for noting the change in the ovaries and uterus. Cows which exhibited estrus were bred either by AI by NS.

In Group I 87.5% exhibited oestrus within 8 to 20 days following treatment. The oestrus discharge was copious in 6 cows and moderate in one cow. In Group II only 50% exhibited oestrus within 10 to 21 day following treatment. The oestrus discharge was moderate to copious in all the four cows.

The persent findings of Group I cows are in agreement with those recorded by Kodagali *et al.,* (1981) and Shams *et al.,* (1991) and Pattabiraman *et al.,* (1986) in cows. The present findings of Group II cows are in agreement with those reported by Sadasiva Rao and Ramamohana Rao (1984) in buffaloes. Where as Slightly higher percentage (59.37) was recorded by Majumdar (1989) in cattle.

Out of the cows bred in Group I 85.71 percent were pregnant while only 50 percent were pregnent in group II. These findings are in agreement with the reports of Kodagali *et al.,* (1981) in Gir cows Bhattacharya and Shanmughasundaram (1982) in crossbred cows and Sadasiva Rao and Ramamohana Rao (1984) in buffaloes. The present study indicate that Inj. Receptal (GnRH analogue) by intramuscular route is an effective treatment in inducing oestrus in anoestrus cows.

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Correlation of Physical Measurements and Production Traits in Red Kandhari Cattle

B.B. GAIKWAD[1], P.G. SAKHARE[2] and K.S. DESHPANDE[3]

College of Veterinary and Animal Sciences,
Marathwade Agricultural University, Parbhani, Maharashtra

In the present study 15 body measurements and 3 economic traits of 60 Red Kandhari cows were analysed to estimate measures of dispersion for body measurements and economic traits, correlations amongst body measurements and correlations and regressions of economic traits with body measurements.

The means of body measurements (cm) were height behind hump 119.48, body length 116.88, hook to pin length 37.77, height at hook bone 119.32, width at hook-bone 37.47, height at pin-bone 110.13, length of tail 96.50, heart girth148.00, height at elbow 69.98, measurement below knee 34.53, length of face 46.40, width of face 21.13 length of ear 28.07, width of ear 14.68,slope of rump 9.45 cm. The economic traits were age at first calving 1443.66 days, lactation yield 558.27 kg. and calving interval 459.91 days.

The correlations amongst most of the body measurements were significant. The correlations and regressions of economic traits with body measurements were non-significant except length of ear with age at first calving and height at elbow with calving interval. The correlations amongst economic traits were also non-significant. The most of the body measurements were similar to Malvi and Nimari cows (ICAR, 1979). Ghafoor (1987) reported body measurements in Red Kandhari which are similar to present measurements.

The significant correlations amongst body measurements were reported in Deoni cattle by Deshpande and Singh (1978). The non-significant correlations of age at first calving, lactation yield and calving interval with body measurements were reported in Jersey cows by Pandya *et al.,* (1986). In ongole cows by Rao and Venkaya (1973) and in Red Kandhari cows by Dhumal (1987). The negative values of regressions of age at first calving, lactation yield and calving interval with body measurements were reported in Red Sindhi cows by Mishra *et al.,* (1978) and in Sahiwal cows by Sudhir Kumar (1978).

Present address:
1. Livestock Development Officer (Extension) P.S. Murud-Janjira Dist. Raigad
2. Professor of Animal Management
3. Professor of Animal Genatics & Breeding

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Clinical Efficacy of Orally Administered Indigenous Preparation (Metrali) In Post-Partum Disorders

L.K. SHARMA

SciTech Centre, 7, Prabhat Nagar, Jogeshwari (W), Bombay 400 102

For a successful and profitable dairy management, fertility of the animal plays a vital role. Diorders of reproductive organs directly impair the fertility and hence economy of the dairy industry. Among the various causes of infertility in animals endometritis is found to be the commonest. Different lines of treatment have been suggested to treat these conditions (Narasima Rao and Kesavamurthy, 1972, Kaikini and Deshmukh, 1984), most of them includes intra uterine preparations for localised and parentral formulations for systemic actions. The present trial was conducted to assess the clinical efficacy of an indigenous formulation administered orally to dairy animals having post-partum disorders.

Dairy animals (17 bufaloes and 4 cows) having clinically diagnosed post-partum ailment were included in the present trial. Indigenous formulation (METRALI*) filled in hard gelatin capsules was dosed at the rate of 50 g per animal for 8 days. Dosing was done by placing the capsules directly into the mouth of the animal at the back on the tongue. Cases in which clinical recovery was not complete, treatment was continued for another 7 days.

Uterine disorders recorded during the present trial were pyometra and endometritis with a history of retention of placenta. (Arthur, 1975). Clinical signs inlcuded copious, whitish yellow coloured muco purulent discharge with foetid odour, flabby and doughy feel of uterus and inflammed vaginal mucus membrane.

Out of 21 animals treated, 19 animals showed reduction in quantity of discharge substantially, within 2-3 days of the treatment. Remaining 2 bufaloes continued to pass traces of purulent discharge with foetid odour even after eight days of treatment. In these cases treatment was continued for another sevendays. All animals showed complete clinical recovery within 15 days of the last treatment. No inflammation of genitalia was observed thereafter and uterine tone returned to normalcy.

Ingredients of indigenous formulation (Metrali) are Tribulus terrestris, Asparagus racemosus, Aristolochia indica, Vernonia anthelmintica, Withania somnifera, Emblica officinalis, Terminalia belerica, Terminalia chebula, Ipomoea digitata, Sphaeranthus hirtus, mercury sulphas, and cuprium sulphas. These ingredients are well documented for their antibacterial, antiinflammatory, antiseptic, emmenagogue, and blood purifying properties (Nadkarni, 1989).

Oral administration of preparations in powder form is either done by mixing in feed or as a drench. In the present study administration of the drug in gelatine capsule was found to be convenient and also ensured proper and complete medication.

* Mycon Pharma, 647, Kasba Peth, Manik Chowk, Pune, 411 011.

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Antibiotic sensitivity Pattern of Cervicovaginal Mucus Cultures of Repeat breeding Cows

GURPAUL SINGH AND CHARANPAL SEKHON

Vety. Poly Clinic Sangrur, Punjab

Efficacy and merits of uterine treatment with antimicrobial drugs has been studied by many workers (Randall 1986, Dholakia *et al.,* (1987), Sharda *et al.,* (1991), Krishnan *et al.,* (1994) and Singh 1994). In this study the cervicovaginal mucus of repeat breeding cows was subjected to in-vitro culture sensitivity test. They were treated with the most sensitive antibiotic and the results after first and following A.I. are presented.

The cervicovaginal mucus of fourty eight repeat breeding cows was collected aseptically during estrous phase were subjected to in-vitro antibiotic sensitivity test using standard procedure recommended by Bauer *et al.,* (1966). The dose and duration of treatment was decided according to the type of infection. The volume of infusion ranged from 50 to 100 ml. Cows which did not repeat after first and following A.I. were diagnosed for pregnancy per rectum after 90 days post A.I.

The growth of bacteria was recovered in 46 (95.83%) out of total 48 samples. The cultures were found sensitive to different antibiotics. The antibiotic sensitivity was maximum for gentamicin followed by chloramphenicol, ampicilin and penicillin. All the cultures were found resistant to oxytetracycline. The less sensitivity to ampicillin, penicillin and resistance to oxytetracycline might be due to their common use in the field. Gentamicin has been reported to be the most sensitive antibiotic against bacteria isolated from the repeat breeders by Dhlakia *et al.,* (1987) and Sharda *et al.,* (1991). Gentamicin and chloramphenicol have been reported as the most effective antibiotics against bacteria isolated from the cervicovaginal mucus of repeat breeding cows by Krishnan *et al.,* (1994).

Repeating Breeding cows treated with Gentamycin 800-1200 mg gave 73.7% conception at first AI and 13.2% in the subsequent AI. Chloramphenicol given at the dose of 2gms resulted in 40% conception at first and 20% conception in the subsequent AI.

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Mummified Female Foetus and Normal Male Calf in a Twin Pregnancy of an Ongole Cow

K. BABU RAO[1], M.S. RAJU[2] and G. NARASIMHA RAO[3]

Livestock Research Station, Lam Farm, Guntur - 522034

The incidence of foetal mummification in cattle is low. Its occurence along with a normal calf in twin pregnancy is rare. (Roberts, 1971). Hence the present case is reported.

An Ongole cow of Livestock Research Station, Lamfarm, Guntur aged 10 years showed signs of parturition along with reddish discharge through vulva and without any visible foetal parts at 275 days of gestation in its 6th calving. Examination per rectum revealed uterus distending into the abdominal cavity with foetal extremities in the pelvic cavity. Vaginal examination showed the cervix to be fully dilated with the foetus in breech presentation without any reflexes. The case tentatively diagnosed as dystocia due to breech presentation of a dead foetus.

By mutational operations and traction, the foetus was delivered, which was female, reddish brown in colour, thin haired, without any signs of putrification and was found to be mummified. Further vaginal examination revealed another live foetus in normal anterior presentation with intact placental membrances. The live foetus was delivered by slight traction after rupturing the placental membrances manually. The calf was found to be a male. The weights of the mummified female foetus and the normal male calf were 13 and 21 Kg respectively and the two placental membranes weighing 4 Kg and 7 Kg were expelled 4 hours after the delivery of the male calf with a gap of 30 minutes.

Rectal examination of the cow on the second day after parturition revealed equal enlargement of both the uterine horns indicating that the present case was bicornual pregnancy. Erdheim (1942) reported that 90 percent of bovine twins were bicornual pregnancies and led to normal birth. Complete involution of uterus was observed within 30 days without any treatment and the male calf was active.

Foetal mummification occurs when a foetus dies without concomitant luteolysis and adequate cervical dilatation (Morrow, 1986). In the present case, the foetus might have died during the last month of gestation and progressed towards mummification, while the presence of the other live foetus might have initiated the parturition towards the end of gestation. Roberts (1971) reported that the mummification of bovine foetus usually affected single foetus but might occasionally involve one or both foetuses in twin pregnancies.

1. Research Officer (Animal Reproduction)
2. Assistant Research Officer (Artificial Insemination).
3. Senior Scientist (Animal Breeding)

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A case Report of Mummified and Normal Foetus in a Bitch

T.N. GANESH[1], S. AYYAPPAN[2], ARCHIBALD DAVID[3], N.N. BALASUBRAMANIAN[4] and S.R. PATTABIRAMAN[5]

Department of Clinics,
Madras Veterinary College, Madras - 600 007.

Foetal mummification is common in swines and felines, less common in cattle and mare but rare in canines (Arthur *et al* 1989). The present paper reports a case of foetal mummies along with one normal foetus in a bitch.

A 3 year old Doberman bitch was presented with a history of mating 45 days back and blood tinged vaginal discharge since one week. The animal was dull and vomiting intermittently.

Clinical examination revealed mild abdominal distension. Haematological examination revealed moderate leukocytosis (Hb 9.5 g/dl; ESR 2 mm/1 hr; PCV 33%; RBC $4.7x10^6$/ml; WBC $23.2x10^3$/ml) Plain radiograph revealed a distended uterus and the case was tentatively diagnosed as pyometra. Medical management of the animal was initiated with antibiotics, fluid and supportive therapy to stabilise the condition prior to surgery. Exploratory laparotomy was decided.

The animal was placed under general anaesthesia with 2.5 percent thiopentone sodium. A low right flank incision was made and exploration of the abdomen revealed a mildly distended uterus with sacculation. Panhysterectomy was carried out as per the routine procedure. Muscles and skin were closed as per standard technique. The wound was dressed and bandaged.

On opening the uterus, one dead normal foetus and 3 mummified foetuses were found (Figure). A small quantity of necrotic tissue and foul smelling sero sanguinous fluid were present within the uterine cavity.

Routine post-operative antibiotic and supportive therapy were given for a week. The dog recovered without any complication. Sutures were removed on the 9th post-operative day.

The present findings of normal sized dead foetus and mummies are in agreement with Roberts (1971) and Arthur et al (1989) who observed that in polytococus species the mummified foetus will not affect the development of normal foetus since maintenance of pregnancy in this species is due to progesterone produced by corpus luteum. The plain radiography and haematology were suggestive of pyometra. However, after surgery the distended sacculated uterus revealed the presence of a normal foetus alongwith three mummies. An unusual case of retained pups and pyometra has been reported by Hjerpe (1961). In bitches foetal death beyond 45 days can lead to abortion or mummification (Freak, 1962). Prabhakar *et al* (1993) also reported foetal mummification along with the presence of normal fetus in two bitches found at whelping.

The present case is placed on record because the radiograph and clinical examination did not reveal the presence of mummified or normal dead foetus. The difference in sizes of the foetus suggest that the mummification and death of the normal sized foetus would have occured at different periods of gestation.

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1. Associate Professor, Department of Clinics.
2. Assistant Professor, Department of Animal Biotechnology.
3. Professor, Department of Surgery.
4. Professor and Head, Department of Surgery.
5. Professor and Head, Department of Clinics.

Figure showing one dead normal foetus and three mummified foetuses

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Modified Uterine Flushing Technique In Repeat Breeders

R.E. NAPOLEAN, D. ANTOINE, D. KATHIRESAN*, T.G. DEVANATHAN** AND S.R. PATTABIRAMAN***.

Livestock Research Station, Kattupakkam - 603 203
Tamil Nadu Veterinary and Animal Sciences University

Apart from ovulatory disturbances and embryonic death, obstructions of the oviduct was found to be the etiological factor in 9 per cent of repeat breeders (Hjerpe, 1961). Therapeutic uterine flushing with normal saline was found to increase the conception without altering the estrous cycle length and damage to the endometrium (Seguin *et al.,* (1974 and Coe, 1984). Considering this, uterine flushing was carried out using a modified technique in Repeat breeders and the effect on conception was studied.

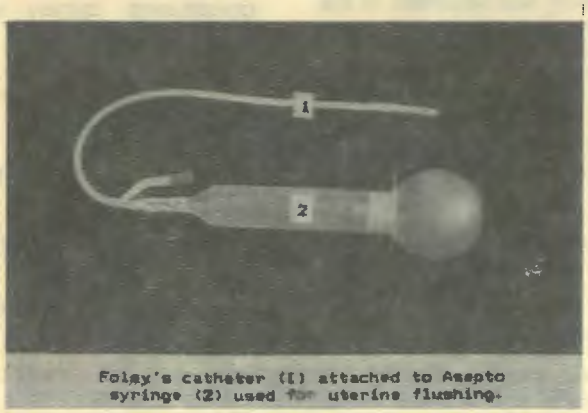
MODIFIED UTERINE FLUSHING TECHNIQUE

Materials required are:

1) Foley's Catheter of 16 or 18 G in size (Fig.) 2) Asepto Syringe of 100 ml capacity (Fig) normally used for flushing the urinary bladder in human practice. 3) 10 cc syringe and glasswares like Beaker and Conical flask 4) 1000 ml of sterile saline solution 0.9 N concentration;

STEPWISE PROCEDURE OF FLUSHING TECHNIQUE

1) The Foley's catheter was introduced into the uterus through a metal stillete and fixed at the base of one uterine horn by inflating the bulb with 8-10 cc of air through a syringe as per the procedure of Elsden *et al.* (1976) employed for non surgical recovery of bovine eggs. 2) Sterile saline solution was aspirated through the nozzle into the Asepto syringe by pressing the rubber bulb fixed at the base of the syringe. 3) The nozzle provided in the Asepto syringe is fixed directly with the Foley's catheter (Figure). Infusion of fluid inside the uterus through the catheter was done by gently pressing the rubber bulb. 4) The quantum of fluid to be infused inside the uterus was assessed by the distension of the uterus. Excessive infusion of fluid was avoided to prevent the endometrial



Foley's catheter (1) attached to Asepto syringe (2) used for uterine flushing.

*Assoc. Prof. E.T. Scheme. **Assoc. Professor and
***Professor & Head, Department of Clinics, Madras Veterinary College, Madras -7

seepage and the resultant poor recovery of the fluid. 5) By slow release of the pressure on the rubber bulb, the infused fluid was collected back into the syringe and then either discarded or collected in a container. 6) Fresh saline solution was taken inside the syringe and flushing was repeated for 3 to 4 times in the same horn. 7) The Foley's catheter was deflated and then introduced into the other horn of the uterus and the bulb was fixed at the base. 8) The flushing procedure for the other horn was repeated with flesh saline solution. 9) After flushing the uterine horns the catheter is removed by deflating the air and the uterus is left undisturbed till the next cycle.

EFFECT OF UTERINE FLUSHING IN REPEAT BREEDERS

Sixteen repeat breeding cows available in Livestock Research Station were utilised for the study in which eight cows were alloted to the experimental group and eight cows served as untreated control group. Flushing of the uterus of cows in experiment group was done on the day of estrum with this modified technique. All the experimental cows and untreated controls were inseminated in the subsequent estrous cycle and the conception rate was compared with the untreated control cows upto three inseminations.

The overall conception rate recorded in the experimental animals following therapeutic uterine flushing was 75 per cent compared to 37.75 per cent in the untreated control cows with an average number of 2.50 and 6.30 services per conception, respectively.

Increased fertility observed in the experimental animals of the present study agrees with the findings of Coe (1984) who reported conceptions in 13 out of 20 repeat breeder cows following uterine flush. Intrauterine infusion of physiologic saline solution has been reported to produce similar results compared with other antibacterial agents (Hjerpe, 1961). However in the present work, infusion of saline with mild pressure through the asepto syringe could have sensitised the endometrium by cleansing and removal of tubal occlusions. Stimulation of endometrium for increased leucocyte infiltration and triggering of uterine defence mechanism could be the other factors responsible as reported by Paisley *et al.,* (1986) for increased fertility in repeat breeders following this modified technique.

Modified technique for flushing of uterus can be performed at the field level with minimum assistance and discomfort to the animal. Uterine flushing can be used for further studies related to the estimation of uterine luminal fluid constituents namely enzymes, minerals and cellular contens.

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An Analysis of Research Work done in Animal Reproduction

A. RAMAMOHANA RAO,
Dean of P.G. Studies (Retd.),
APAU, Hyderabad.

Research work on different areas of animal reproduction are being carried out for several years by the various veterinary colleges and research institutes in India. It would be of great benefit if the research work so far done is analysed to identify the thrust area most relevant to the present requirement of the country. This will also help to avoid repetetive and purely accademic research work. With this objective in view 841 research articles on various aspects of animal reproduction published during 1981-95 in the Indian Journal of Animal Reproduction the official organ of the Indian Society for the study of Animal Reproduction exclusively publishing articles on animal reproduction since 1981 were taken as the source material for analysis.

The articles related to female reproduction are classified as 1. Physiology including anatomy and histology, 2. Controlled breeding techniques, 3. Embryo transfer technology, 3. Infertility, 4. Clinical diagnosis, 5. Obstetrics and 6. Miscellaneous.

The articles related to male are classified as 1. physiology 2. Infertility, 3. Semen collection and production 4. Semen analysis 5. Semen preservation and 6. Fertility and breeding soundness.

Species wise analysis revealed that the articles relating to cattle, buffaloes, goats, sheep, pigs and others are 47.6, 26.2, 14.5, 5.5, 2.5 and 3.8 per cent of the total number of papers published respectively. More or less the same trend is found in both sexes.

Analysis of publication on female reproduction revealed maximum work on physiology (42.3%). Work done on infertility and obstetrics are 17% each. Controled breeding was given the least importance (3.4%). There is not much of difference between the species except that more work seems to have been done and published in Buffaloes, sheep and goat than in cows in the area of obstetrics.

Analysis of work done on male reproduction revealed that the maximum work (38.1%) was on preservation semen. The next frequently dealt area was semen analysis (26.6%). Twelve per cent of work was on fertility while 9.6% of work was on infertility. Unlike in female only 10.6% of work was related to Physiology. The least (3.2%) was on semen production and collection. There was not much of difference between species but very limited (only 3.3%) work on infertility in Buffalloes was done in comparison to 16.3% in cows.

In female reproduction controled breeding techniques involving sychronisation of estrus etc. in cattle and buffaloes are relatively less. It is desirable to do more research on controled breeding techniques to augment fertility and fecundity in bovines and particularly in buffaloes. Embryo Transfer Technology has emerged as one of the important areas of research in the last decade and the number of articles published appeared to be encouraging. The research work on ETT are more in goats and least in buffaloes. In tropical countries several forms of infertility has been reported, but fundamental studies on the role of trace minerals and endocrine, disturbance are comparatively few. Research articles based on clinical conditions such as heat detection, pregnancy diagnosis, retained placenta abortions etc., which have great relavance to field veterinarians are inadequate. More emphasis should be given for this area which will go a long way to improve the productivity of the animals.

In male reproduction the number of publication is comparatively low. They constituted

only 26% of the total articles published. Nearly 65% of the work were on semen evaluation and semen preservation. A lot of research appears to be on this area which unfortunately repetetive and does not add much to the available knowledge. The same type of work was repeated in different species or breeds of same species. Information on infertility in breeding bulls appears to be scanty although this is a very important area to obtain good fertility in the field.

It is concluded that in female reproduction thrust should be given on clinical research, controled breeding to augment fertility and ETT. More work on buffaloe is required. In male emphasis should be given on infertility problems which is very vital for the implementation of A.I. Programme. Overall research should be reoriented to generate information on problems encountered in the field