IJAR. 16(1), 1995; 1-4

Modern Trends in Gynaeco-Clinical Therapy for Augmenting Bovine Fertility

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Dr. C.R. SANE ORATION LECTURE: AT 12TH CONVENTION OF ISSAR, PKU, AKOLA - JANUARY 1995

With the expansion of dairy industry, full genetic potential in terms of milk production from the cow / buffalo can be exploited only when the reproduction is normal. A normal fertile cow should calve first at two years of age and then at every 12 months with a minimum service period of 85 days post-partum, 1.6 services / conception and should maintain a full term pregnancy.

The re-establishment of regular oestrous cycle after parturition in cows / buffaloes is delayed for a variable period of time. The influence include genetic, environment, nutritional status, milk yield, parity, breed, calving difficulties, postpartum diseases, ovarian disorders and inadequate amount of gonadotrophins. In recent years considerable attention has been focussed on reproductive endocrinology as a means to identify specific problems and to adopt therapeutic measures for augmenting bovine fertility.

The gynaeco-clinical therapy includes correction of functional disorders of gonads, synchronization of oestrus and uterine infection. In modern trends progestogens, gonadotrophins and prostaglandins are used for augmenting bovine fertility.

1. Therapy for functional disorders of gonads:

(1) Progestogens:

Functional anoestrus due to ovarian inactivity is considered to be the most expensive and frustrating problems associated with buffalo production in India. Oestrus synchronization means pharmacological control of oestrous cycle by using exogenous endocrinological substrates (Hormones or their analogues) at various phases of oestrous cycle.

The effectiveness of progestogens in regulating and synchronizing oestrous cycles has been extensively investigated in cows and in buffaloes probably by the removal of negative feed back inhibition of the responsiveness of the pituitary to gonadotrophin-releasing hormones. However, treatment combinations involving short term use progesterone and gonadotrophin or progestogen and prostaglandin have produced better synchrony of oestrous response and higher fertility than with progestogen alone.

The administration of progestogen, a synthetic analogue of progesterone mimics the action of natural progesterone. The administration can be done by way of feeding, daily i/m injections or inserting long active depot preparations under the skin or in vagina of the cow / buffalo. PRID (Progesterone releasing intravaginal device) is a silastic coil impregnated with 1.55 g progestogen having gelatin capsule of 10 mg Oestradiol benzoate attached to the inner surface. On removal of coil on 10th day A.I. is done at 48 to 72 hours. Ear implant (18 x 3mm) pessary in polypropylene sheath containing 6 mg norgestomet is implanted by special syringe subcutaneously on the outer portion the ear for 10 days. At the time of implant 6 mg oestradiol valerate and 3 mg norgestomet are injected i.m.

(2) Gonadotrophins:

a) Pregnant Mare Serum Gonadotropin (PMSG): The delay to first post-partum oestrum is the most-vital factor responsible for reproductive inefficiency of dairy animal. It is presumed that early resumption of normal ovarian function is limited by deficiencies in hypothalamic or pituitary functions, resulting in failure of release

of pituitary hormones which are responsible for initiation of post partum ovarian activity. PMSG will stimulate follicular growth in the ovaries, producing endogenous estrogen which will exert positive feed back on the anterior pituitary function and in term, the ovarian cyclicity in early post partum period. PMSG has been used by many research workers for superovulation in cows for embryo transfer. Importance of high plane of nutrition was emphasised to get good response to PMSG and indicated that 5 out of 14 females ovulated on low plane of nutrition v/s 17 out of 17 females on a normal level of nutrition.

Delayed sexual maturity of buffalo heifer continues to be a major problem, even though the animals achieve the desired chronological age of puberty as well as normal body weight. The age of puberty can be lowered in buffaloes with better management and feeding. Hormonal treatment is fast becoming a common method of bringing anoestrus animals into regular cyclicity. PMSG has been successfully used in cattle and buffaloes at an age at which it was economically, desirable to have a endocrinic process initiated.

b) Human chorionic gonadotrophin (HCG): Luteinizing Hormon (LH): Clinically, quite often fairly well developed follicles are palpated mostly in one or both the ovaries and ramains without ovulation especially in repeat breeders. If the condition persists for a long time it may be followed for a cystic ovarian degenaration.

Delayed ovulation is one of the major causes of repeat breeding in crossbreds as only 66% of the cows were observed ovulating within 24 hrs. of insemination, As LH surge plays an important role in ovulation and luteinization of follicle, its treatment at the time of insemination has resulted in increased onception rate.

c) Gonadotrophic Releasing Hormone (GnRH): Early establishment of cyclic ovarian activity in post partum dairy cows is desirable as this improves reproductive efficiency. It has been observed that the post-partum problems frequently delay the onset of cycling by some how affecting hypothalamic hypophysial gonadal axis in some unknown way. Administration of GnRH in cows stimulates the release of endogenous LH and FSH. Ovulations can be induced and normal oestrus cycles initiated when dairy cows were treated on day 13 post-partum with GnRH. Treatment of dairy cows with GnRH also reduced the incidence of ovarian cysts. It has become a routine practice to administer GnRH in early post partum cows in attempt to hasten early resumption of cyclic ovarian activity. It is observed that factors like season, nutrition suckling, ovarian picture at the time of drug administartion, methodology of drug administration influenced the success of GnRH treatment.

d) Clomiphene citrate: Clomiphene citrate has action to stimulate hypothalamopituitary axis to release gonodotrophin releasing hormone which then activates pituitary for secretion of gonadotrophins. A non-hormonal combination of two isomers of Triphenyl Ethylene compound (Clomiphene) each containingCis-clomiphene citrate 180 mg and Trans-clomiphene citrate 120 mg. dissolved in 500 ml of water and administered daily as a drench for a period of 5 days resulted induction of oestrus in cow and buffaloes.

e) Active immunization by steriod-link-protein: The new technique of steriod immunization was investigated in Thailand as a possible means of stimulating ovarian function in the swamp buffloes. The principle of immunization is that a steriod immunogen induces the production of antibodies to partially neutralize steriods involved in hypothalamic-pituitary feed backs and thus allow more gonadotrophin release. Hormonal imbalance in ovarian function may be corrected by such immunization and may result in ovulation or even multiple ovulations. Enhancement of female fertility was increase by 30% in non-pregnant buffaloes with active CL by means of synchronisation and A.I. and 24% in non-pregnant subfertile by means of active immunization. Steriod-Link-Protein in a system of active immunization certainly seems to be on appropriate biotechnology to enhance fertility of village buffaloes.

3) Prostaglandin ($PGF_{2\alpha}$):

Over the past 10 years evolution has gradually developed of the use of prostaglandin $F_{2\alpha}$ for synchronizing oestrus and thus facilitating and A.I. programme, particularly under the circumstance when genetic progress is aided by cross-breeding. The problem of silent oestrus especially in buffaloes is very frustating and use of prostaglandin is a boon in such conditions although it remains to be determined whether this technique can be readily applied under the farming systems prevailing at present in India.

II. Therapy for Unterine Infection:

An ideal therapy for uterine infection should: i) Eliminate bacteria from the uterus, ii) not inhibit the normal uterine defence mechanism (UDM), iii) not cause further adulteration of milk or meat for human consumption.

a) Antibiotic therapy: In-vitro antibiotic sensitivity tests were for the first time discovered and designed in 1947 by using single paper discs. The evaluation of chemo-therapeutic agents against genital tract infections, based on their prior *invitro* sensitivity results, has received considerable attention during recent years mainly due to their reliability and economy.

b) Success of IU treatments: Many factors contribute to the diminished efficacy of most of the commenly used IU drugs. The postpartum bovine uterus is an anerobic environment, making the aminoglycoside group of antibiotics (gentamycin, Kanamycin, streptomycin, neomycin) ineffective because they require oxygen for their activity. During the early post-partum period, many organisms in the uterus are capable of producing enzymes that inactivate or degrade antibiotics viz, pencillinase. The presence of pus and organic debris in the uterine fluids could potentially inhibit drugs such as the sulfonamides, aminoglycosides, and nitrofurazone. During the early postpartum period and in cows with endometritis, the absorption of many drugs is greatly diminished. When absorption of drugs is low, therapeutic levels in the deeper layers of the uterus and other parts of the genital tract are not likely to be achieved.

Irritating IU antibacterials should not be used in postpartum cows or cows with pyometra, Irritating solutions cause a necrotizing endometritis and leucocytic exudation in cows with normal uteri or with mild endometritis. This may be beneficial, although experimental evidence is lacking, irritating solutions have no effect on the interval between cycles if they are infused during midcycle, or at oestrus but they prolong the interval if given on days 16 to 19.

The preponderance of literature reviewed, supports the conclusion that there is only an occasional beneficial effect of using antibiotic and antibacterial therapy for uterine disorders in the postpartum period. Cows with systemic involvement often respond to antibiotic therapy as a life saving procedure or for reducing the deleterious effects of toxemia or septicemia on milk production. Routine intrauterine antibiotic therapy in cows without histories of post-partum disorders cannot be justified nor can the use of intrauterine antibiotics, post-insemination in repeat breeding cows. The prophylactic application of intrauterine tetracycline drungs in cows with retained fetal membrane (REM) has been reported to be beneficial or detrimental to fertility. Despite treatments with a variety of intra-utrine drugs, the fertility of cows with REM is often less than that of unaffected cows.

c) GnRH in uterine infection: The variable results following GnRH treatment in post-partum cows may be largely due to the ovarian changes, endocrine events and uterine infection patterns. Therefore, it is reasonable to assume that the interaction of uterine infection and prior follicular development may influence response to GnRH treatment in post-partum cows.

In a study on 50 Holstein lactating cows treated with 100 ug GnRH on day 15 post-partum, check-up of ovarian activity on per rectal examination and ultra-sonography and endometrial swab culture suggested that intrauterine infection delayed post-partum follicular development and was detrimental to resumption of ovarian activity.

d) Prostaglandin $F_{2\alpha}$ Therapy for postpartum uterine disorders: The rational of

using $PGF_{2\alpha}$ for post-partum uterine infection is : i) $PGC_{2\alpha}$ induced luteolysis, decreases progesterone inhibition of the uterine defence mechanism (UDM) ii) estrogen production, which follows luteolysis, stimulates the UDM; iii) $PGF_{2\alpha}$ may stimulate contractions that aid in the expulsion of uterine lochia, pus or other contents; and iv) $PGF_{2\alpha}$ may have a stimulatory affect on phagocytosis by uterine leucocytes.

Evidence from experimental and field trials demonstrates the effectiveness of $PGF_{2\alpha}$ treatments, showing it to be the most effective therapy for pyometra. One $PGF_{2\alpha}$ injection resulted in negative bacterial cultures and normal clinical findings in approximately 90% of cows with chronic endometritis (pyometra). Cows that failed to respond completely after one $PGF_{2\alpha}$ treatment often recovered after a second injection 10 to 14 days later. Any advantage from supplemental intra-uterine or systemic antibiotic therapy has been only rarely demonstrated. Cows receiving cloprostenol had 22% better first-service conception rates, 21% fewer inseminations per pregnancy and 15% improvement incalving-to-conception interval. When comparing the percentage of treated cows that become pregnant within 85 days of calving, a "must" if 300 days calving interval is to be maintained, it was found that 60.9% of the cloprostenol treated group achieved this goal vs 38.2% in the intra-uterine treated group, with an improvement of 59.4%. These are the "economically importent" parameters and hence clinical recoveries without corresponding improvements in fertility are of little value.

In terms of feritility, the therapeutic and prophylactic use of $PGF_{2\alpha}$ and its analogues in the post-partum period consistantly show results equal to or better than IU antibacterial therapy. Because $PGF_{2\alpha}$ therapy has the advantages of systemic vs intrauterine administration without affecting or tainting the milk yield, it should be recommended as the initial treatment regimen in post-partum uterine disorders. It is likely that extended intervals from parturition to insemination either through choice or because of estrus detection failures, are more responsible for long parturition-to-conception intervals than are uterine infections. Inducing ostrus with $PGF_{2\alpha}$ not only has therapeutic value for uterine infections,but it also shortens the interval from parturition to each successive estrus following the induced estrus. Shortening the interval from parturition to first or later insemination by five to ten days with $PGF_{2\alpha}$ therapy would decrease the calving-to-conception interval by a similar time regardless of any beneficial effect on uterine infections or increased oestrus detection efficiency.

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Study of Ovarian Steroids During Post-Partum Period of Surti Buffaloes in Relation to Suckling and Milking Practices

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ABSTRACT

Circulating levels of progesterone (P_4) and estradio 17 beta (E_2) were estimated from blood samples of suckled and milked farm born Surti buffaloes during post-partum period. Average levels of progesterone and estradiol-17 beta were lower in suckled buffaloes compared to milked ones (0.41 vs 0.52 ng / ml progestrone and 85.30 vs 117.49 pg / ml estradiol 17 beta). Decrease in progesterone concentration immediately after calving was sharp in milked buffaloes in contrast to nonspecific fluctuations in suckled group. Concentration of estradiol-17 beta increased immediately after calving in milked animals against decrease in suckled buffaloes. A specific short lasting pre-estrus rise in progesterone concentration was noticed around day 46 and 24 in suckled and milked buffaloes respectively. Thus study revealed that suckling influences profile of ovarian steroids reflecting ovarian activity during post-partum period.

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Long intercalving period in buffaloes is one of the constraints comming on the way of their reproductive performance and thereby affecting their productivity. Suckling, apart from other factors, plays an important role in regulation of post-partum ovarian activity, hence an attempt was made to know the effect of suckling on ovarian steroids during postpartum period to effect of ovarian activity.

MATERIALS AND METHODS

Normally calved 12 farm born Surti buffaloes, managed under standard management and feeding practices, were selected for this study. These were put into two groups - six animals in each group-I (suckling group) - calves were allowed to suckle twice a day ad-lib and group-II (milking group) - calves were weaned at birth and buffalo were milked twice a day. Jugular blood samples were collected at pre planned stages - immediately after calving, one hour after milking or suckling, immediately after placental expulsion and then every 48 hours till first heat post-partum. Serum was separated and stored at - 20°C till analysed.

Progesterone (P_4) was estimated by solid phase RIA as per the method of Kubasik *et al.* (1984). The sensitivity of the asay was 0.05 ng / ml, inter and intra assay variations were 10, 6.6, 7.2 and 8.4, 7.5 and 5.8 per cent respectively for low, medium and high concentrations. Estradiol-17 beta was estimated as per Robertson *et al.* (1979) with the sensitivity of 1.4 pg / ml. Inter and Intra assay variations were 2.7, 3.5, 4.5 and 15, 5 and 5 percent respectively for low, medium and high concentrations. The results were analysed statistically to know the variation between the groups and between the stages within the group as per Snedecor and Cochran (1971).

RESULTS

Variation in the serum P_4 between the stages within the group were statistically significant for both the groups ($P<0.05$). Overall mean concentration for serum P_4 was 0.41 ng / ml (range 0.24±0.04 to 1.17±0.15 ng / ml) and 0.52 ng / ml (range 0.27±0.04 to 1.09±0.12 ng / ml) respectively for group-I and II. The fluctuations recorded for serum P_4 was almost same in both the groups, however the decrease in the level, specifically during early post-partum period (upto 2 day PP), was faster in animals of group II (milking group), than that of animals of group I (Fig. 1 - $P<0.05$) by 'T' test). There was a specific short lasting rise in P_4 concentration at around day 46 (1.17±0.15

ng / ml) and day 24 (1.09±0.12 ng / ml) postpartum of group I and II respectively (Fig. 1).

Like P_4, Serum E_2-17 β also showed significant stage variations within the group ($P<0.01$). The average concentrations of serum E_2-17 Beta was 85.30 pg / ml (range 22.06±4.16 to 120-.59±27.31 pg / ml) and 117.49 pg / ml (range 42.46±10.82to 142. 13±29.15 pg / ml) for group I and II respectively. Immediately after calving i.e. upto 2nd day post-partum, the levels of E_2-17 beta decreased in the animals of group-I while it increased in other group (Fig. 2). There after the level remained oscillating and towards first heat post-partum it started increasing (from day 40 and 18) reaching peak by day 57 and 29 post-partum respectively for group I and II (Fig.2).

DISCUSSION

Circulating levels of estradiol-17 beta and progesterone during post-partum period reflects the reestablishment of ovarian activity, influencing post-partum reproductive performance. This attempt was made in view to see the influence of suckling and milking practices on circulating levels of estradiol-17β and progesterone during post-partum period of Surti buffalo.

Variation in circulating P_4 level reflected the ovarian activity (Jainudden *et al.* 1983), and also regulate length of estrous cycle (Mc Donald, 1980) as main source of P_4 is cropus luteum. The trend and levels of P_4 observed in the present study (Fig.1) are comparable with that of Pahwa and Pandey (1983) for Murrah buffaloes, Perera *et al.* (1987) for Shrilankan buffaloes and Jainudeen *et al.* (1981) for Swamp buffaloes.

There was no significant difference in the levels of P_4 as well as the patterns between the groups, however the levels were lower in the animals of the group I. This indicates that suckling has no effect on P_4 concentration. Dunlap *et al.* (1981) and Suzuki and Sato (1981) also reported same type of findings. A specific rise in P_4 level was recorded by day 24 and 46 in group I and II respectively. This rise was short lasting, as the level came to base immediately resulting the estrus (Tiwari, 1989). Such type of pre-estrus rise have been reported in Murrah (Pahwa and Pandey, 1983), Egyptian (El-Belely *et al.* 1988) and Swamp buffaloes (Kamonpatana *et al.* (1981). This pre-estrus rise in P_4 is considered to be ovarian origin - luteinised follicle (Lamming and Bulman, 1976) essential for priming postpartum ovary for action of gonadotrophin (Pahwa and Pandey, 1983) and represent the reestablishment of ovarian activity (Jainudeen *et al.* 1983).

The averaged levels of E_2-17β reflecting follicular development, in the present study were comparable with the results of Devaraj (1982) in Surti buffaloes, but were slightly higher than those reported for Murrah buffaloes (Pahwa and Pandey, 1983 and Lohan *et al.* 1987) for Nili Ravi buffaloes (Samad *et al.* 1988), for Swamp buffaloes (Kamonpatana *et al.* 1978) and for Egyptian buffaloes (El-Belely *et al.* 1988). This difference may be considered as breed characteristics.

In animals of group I, the levels of $E_2$17β dropped down immediately after parturition where as as it increased in the animals of group II and remained high upto day-2 (Fig.1, $P<0.05$) by 'T' test). This may be due to the effect of suckling stimulus as indicated by Bellin *et al.* (1984). Same type of observations have been reported by Pahwa and Pandey (1983), Lohan *et al.* (1987) and El-Belely *et al.* (1988). Higher level of Estradiol-17 beta and rapid fall in progesterone concentration during early post-partum period of animals of group II (Fig. 1 & 2) favours estrogen induces luteolysis (Wiltbank, 1966).

Animals of group I showed wider fluctuations in the levels o estradiol-17β comapred to the animals of group-II. This may be due to waves of follicular development which was confirmed by rectal palpation of these animals. More than 75 per cent of the animals in group I had three waves of follicular development, reaching to final maturation (in group II) which was reflected by serum E_2-17β (Fig.1). This gives support to the findings of Eduvie *et al.* (1985) that suckling stimulus interferes the final stage of follicular maturaiton.

Thus, overall the study reveals that suckling stimulus affects the functional aspects of ovary in post-partum period.

Fig.1 SERUM PROGESTERONE (ng/ml) IN SUCKLED AND NONSUCKLED SURTI BUFFALOES

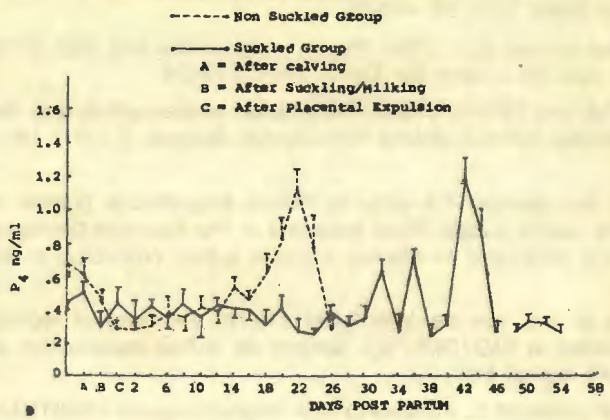

Fig. 1

Fig. 2: SERUM E_2 17B (pg/ml) IN SUCKLED AND MILKED BUFFALOES

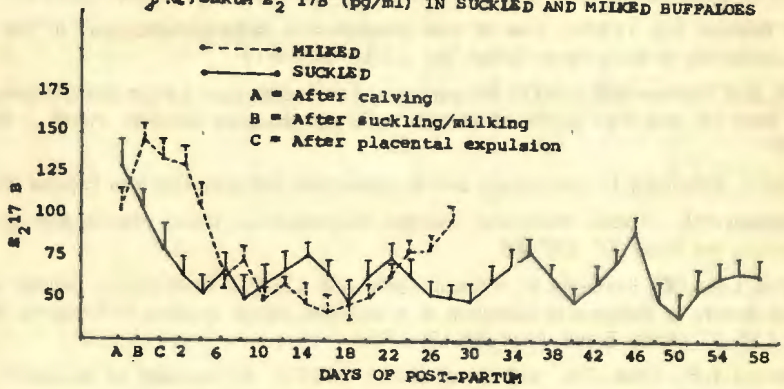

Fig. 2

REFERENCES

Bellin M.E., Hinshelwood M.M., Hanser E.R. and Az.R.L (1984). Influence of suckling and side of corpus luteum or pregnancy on folliculogenesis in post-partum cows. Biology of Reproduction *31:* 849-55.

Devaraj (1982). Blood serum profile in calves and post partum buffaloes (Surti breed) with associated peridata to reproductive efficiency, Ph.D. Thesis, submitted to Gujarat Agricultural University, Anand, Gujarat State, India.

Dunlap S.E., Kiser, T.E., Rampack G.B., Kraeling R.R. and Thompson F.N. (1981). Effect of suckling on cortisol, progesterone and luteinizing hormone in post-partum beef cows. Theriogenology *16* (2) : 185-93 (Anim. Breed. Abstr. *51:* 150.

Eduvie L.O, Njoku, C.O., Molokwu, E.C.T., Oseri D.I.K. and Addo P.B. (1985). Ovarian activity and changes in suckled and nonsuckled cows during post-partum period. Journal of Animal production Research *5* (2) : 159-74 (Anim. Breed. abstr.*55:* 4956).

El-Belely W.S., Zaki K. and Grunert G. (1988). Plasma progesterone and total estrogen in buffaloes around parturition (Bubalus bulablis) J. Agric. Sci. Camb *111* : 519-24.

Jainudeen M.R., Bongso, T.A. and Tan H.S. (1983). Post-partum ovarian activity and uterine involution in suckled swamp buffalo. (Bubalus bubalis). Animal Reproduction Science *6* (3) : 181-90 (Anim. Breed. Abstr. *51 :* 2801).

Jainudeen M.R., Tan H.S. and Bemgso T.A. (1981). Plasma progesterone profiles in relation to post-partum ovarian activity in the swamp buffalo. Paper presented at 2nd Research Coordinated Research Programme on the use of nuclear techniques to improve domestic buffalo production in Asia. March 2-6, Banghok, Thailand.

Kamonpatana M., Schams D. and Van dle. Wiel D.F.M. (1978). Problems of reproduction in female Swamp buffalo. Paper presented at FAO / SIDA / GOI Seminar on buffalo reproduction and artificial insemination. December 4-15 NDRI, Kamal, India.

Kamonpatana, M., Virakul P., Lohachit C., Kunawongkrit, A. Nagramsuriyaron (1981). LH levels during pregnancy of buffaloes (Bubalus bubalis). 2nd RCM of Co-ordinated Research Programme on the use of nuclear techniques to improve domestic buffalo production. Asia. March 2-6 Bangkok, Thailand.

Kubasic N.R., Hallauer G.D. and Brodows R.G. (1984). Evaluation of direct solid phase Radioimmuno assay for progesterone, useful for monitoring luteal function. Clinical chemistry *30 (2)* : 284-86.

Lamming G.E. and Bulman D.C. (1976). Use of milk progesterone radio-immunoassay in the diagnosis and treatment of subfertility in dairy cows. British Vet. J. *132:* 507-517.

Lohan I.S., Kaker M.L. and Razdam M.N. (1987). Pre partum and immediate post-partum blood plasma progesterone, Estradiol-17 Beta LH and FSH profile of Murrah buffaloes (Bubalus bubalis). Indian J. Anim. Repord. *8* (2) : 121-26.

Mc Donald L.E. (1980). Veterinary Endocrinology and Reproduction 3rd edn. Lea and Febiger, Philadelphia.

Pahwa G.S. and Pandey R.S. (1983). Hormonal changes in postpartum blood plasma and milk of buffaloes (Bubalus babalis). Ani. Prod. *37:* 237-46.

Perera B.M.A.O., Silvia L.N.A.DE., Kuruwita V., Y.Karunaratne A.M. (1987). Post-partum ovarian activity, uterine involution and fertility in indigenous buffaloes at a selected village location in Sri-lanka. Animal Repord. Sci. *14* (2) : 115-27 (Anim. Breed. Abstr. *56* (2) : 753.

Robertson R.D, Richard H.P., Peter C.W. and Douglas M.S. (1979). Assessment of ovulation by ultrasound and plasma determination. Obstetrics and Gynaecology. *54* (6) : 686-91.

Samad H.A., Hamid A. and Rehman N.V. (1988). Plasma estradiol-17 Beta concentration during the estrous cycle of NiliRavi buffaloes. In 11th Intrnational congress on Animal Reproduction" at A.Z. University College. Dublin Ireland. June 28-30, 1988. Vol.2 (Anim. Breed. Abstr. *56:* 7456.

Snedecor G.W. and Cochran W.G. (1971). Statistical methods, 6th Edn. The IOWA State University press, Ames IOWA U.S.A.

Suzuki O. and Sato M. (1981). Effect of suckling on growth of beef calves and post-partum reproductive performance of the dams. Bulletin of the National Grassland Research. Institute Japan (1981). No.20, 145-153 p (Anim. Breed. Abstr. *50:* 3113.

Tiwari (1989). Study on reproductive performance of post-partum Surti buffalo alongwith blood profile (Hormonal and Biochemical) in relation to suckling and non-suckling practices. M.Sc. Thesis submitted to Gujarat Agril. University, Anand, Gujarat, India.

Wiltbank J.H. (1966). Modification of ovarian activity in bovine following injection of estrogen and gonadotrophin. Ovarian regulatory mechanism. J. Reprod. Fert. Suppl. 1:1

IJAR. 16(1), 1995; 9-11

Endocrine Profile During Luteolysis Induced By Low Dose of Cloprostenol In Crossbred HF Cows And Heifers

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ABSTRACT

Cloprostenol at a dose rate of 100 µg per animal resulted in complete luteolysis and onset of behavioural estrus incrossbred HF cows and heifers. The plasma hormonal profile of FSH, progesterone and estradiol 17β in treated cows and heifers were similar. The delayed luteolytic response in heifers and endocrine profile in relation to higher dose of synthetic $PGF_{2\alpha}$ or natural luteolysis in cattle was discussed.

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The response of the corpus luteum to exogeneously administered prostaglandin $F_2\alpha$ ($PGF_{2\alpha}$) differ between species (Niswender *et al.* 1985) as well as within a species (Walton *et al.* 1987) depending upon reproductive status and nature of the chemical used. The hormonal profile either during natural or cloprostenol (synthetic $PGF_{2\alpha}$) induced estrus in boviness was found to be similar (Glencross and Pope, 1981) but, there appears to be difference in luteolytic response to different prostaglandins (Godfrey *et al.* 1989). Moreover, the onset of fertile estrus and subsequent conception rate after cloprostenol or carboprost tromethamine induced luteolysis in rural cattle and buffaloes was found to be dose dependent (Narayana and Honnappa, 1986). Keeping these in view, the luteolytic efficiency of low dose of cloprostenol in cross bred HF cows and heifers was determined.

MATERIALS AND METHODS

Cross bred HF cows (n=6) and heifers (n=6) having palpable corpus luteum were chosen and their jugular vein was cathatrized. The patency of the cathater was maintained with heparin (5iu/ml). All the animals received a single intramuscular injection of cloprostenol (Cooper Animal Health Care, U.K) at a dose rate of 100µg/animal. Blood samples were drawn at every2hr interval begining from few hrs before the treatment till 48 hr after the onset of estrus (day=0). All the animals were closely observed and the onset of estrus was detected based upon the expression of behavioural signs like "standing to be mounted". At the onset of estrus the animals were per-rectally examined for the presence or absence of corpus luteum. The blood samples were centrifuged at 1000 x g for 10 min and the seperated plasma were stored at O°C for pending analysis.

Radioimmunoassay (Ria) of Hormones: The plasma progesterone and estradiol - 17β were twice extracted with hexane and di-ethylether respectively. The RIA of progesterone and estradiol - 17β were carried out in duplicates by using specific antibodies according to standard methods described (Narayana and Honnappa, 1986) and validated earlier (Prakash, 1990). The inter - and intraassay co-efficient of variation for both the

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RIA's were less than 12 per cent. The plasma FSH concentration in samples collected at every 2 hr was measured by solid phase oFSH RIA according to Murthy *et al.* (1989) using oFSH antibody (1:20000) and bFSH as standard (USDA - FSH - BP3). Both the intra - and interassay co-efficient of variation was less than 15 per cent. The 'method blank' carried out with distilled water did not had any effect on respective RIA dilution curve.

Student 't' test was applied to compare the various values obtained from two groups of animals. All the values were expressed as mean.

RESULTS AND DISCUSSION

The onset of estrus occured in treated cows and heifers at 48±4 hr and 108±6 hr respectively. There was no difference either in duration of "behavioural estrus" or the appearent symptoms of estrus between the two groups. There was no palpable corpus luteum on both the ovaries in all the treated animals on day 'O'. The plasma progesterone concentration at the onset of estrus was 0.60±0.15 ng / ml in cows and 0.76±0.2 ng / ml in heifers and these values did not singnificantly differed (P>0.05). At the onset of estrus the plasma estradiol - 17β concentration was 19.18±2.72 pg / ml in heifers and 21.98± 1.57 in cows and these values did not significantly differed (P>0.05). The basal level of estradiol - 17β in both the groups was similar. There was no change in either the pulsatile pattern or the amplitude of FSH concentration between the two groups during estrus. The peak FSH concentration (>60 ng / ml) in both the groups were found 2-12 hr before the onset of estrus. The basal FSH concentration during estrus was 9.27±0.75 ng / ml in cows and 8.70±1.20 ng / ml in heifers and these values did not differed statistically P>0.05).

Based on the plasma ovarian steroid hormone concentration, it is evident that cloprostenol at a dose rate of 100βg / animal can bring complete luteolysis in both cows and heifers. However, delayed luteolytic response was observed in heifers. Similar observation was also made even after the administration of cloprostenol or luprostiol at a dose rate of 500 µg / animal in Brahman cows and heifers (Godfrey *et al.* (1989). O'Shea *et al.* (1989) observed difference in cellular composition of cyclic corpus luteum of the cows and perhaps this explains the difference in luteolytic capacity of $PGF_2\alpha$ in cows and heifers. The luteolytic dose employed in the present study did not influenced either the pre-estrual peak or post-estrual basal FSH concentration in both the groups. In addition to this, cloprostenol dose employed in the present study did not differed in terms of luteolytic ability or induction of estrus either with higher dose rate of cloprostenol or with natural mechanisms (Glencross and Pope, 1981).

Acknowledgement: We are grateful to Coopers Animal Health care, U.K and United States Department of Agriculture (USDA) for kind gift of hormones.

REFERENCES

Glencross, R.G. and Pope, G.S. (1981) : Concentration estradiol - 17β and progesterone in the plasma of dairy heifers before and after cloprostenol induced and natural luteolysis and during pregnancy. Anim. Reprod. Sci., *4:* 93-106.

Godfrey, R.W; Gathri, M.Y; Neuendorff, D.A. and Randel, R.D. (1989) : Evaluation of luteolysis and estrus synchronization by a prostaglandin anàlog (Luprostiol) in Brahman cows and heifers. J. Anim. Sci., *67:* 2067-2074.

Murthy, G.S; Lakshmi, B.S. and Moudgal, N.R. (1989) : Radioimmunoassay of polypeptide hormones uding immunochemically coated plastic tubes J. Biosci., *14:* 9-20.

Narayana, K. and Honnappa, T.G. (1986) : A dose response study with two prostaglandin F_2a analogus, Carboprost tromethamine or cloprostenol in the induction of estrus and a double blind clinical trial of carboprost tromethamine in rural buffaloes and cattle. Ind. Vet. J., *63:* 191-196.

Niswender, G.D; Schwell, R.H; Fitz, T.A; Farin, C.E. and Sawyer, H.F. (1985) : Regulation luteal function in domestic ruminants. Rec. Prog. Hormone. Res., *41:* 102-142.

O'Shea, J.D; Rodgers, R.J. and D'Ocehio, M.T. (1889) : Cellular composition of the cycliccorpus luteum of cow. J. Reprod. Fertil., *86:* 483-487.

Prakash, N. (1990) : The effect of Malathion on plasma concentration of 17β-estradiol and acetylcholinesterase and progesterene and conception rate in dairy cattle. MVSc thesis. Fac. Vet. Pharmacol, Agricultural Sci. Univ.

Walters, J.S ; Veenhaizen, L.P. and King, G.J. (1987) : Relationship between time of day, estrus behaviour and pre-ovulatory LH surge in HF cows after treatment with cloprostenol. J. Dairy. Sci., *70:* 1650-1663.

IJAR 16(1), 1995; 12-14

Comparative efficacy of PMSG and FSH-P on superovulation response of crossbred cows

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ABSTRACT

Comparative effectiveness of two conventionally used gonadotrophins PMSG and FSH-P for inducing multiple ovulation in crossbred cows was studied.

Cows (11), 5-11 years of age, upto 8 lactation and above 60 days post partum were selected based on their regular estrous cycle performance to receive either 40 mg FSH-P (Schering Corp., USA) or 2500 IU PMSG (Folligon, Intervet, Holland). Treatment in both the groups was started on day 11 of the natural estrous cycle. In first group, 40 mg FSH-P was given over 4 days in decreasing dose at 12 hr apart. In second group PMSG was given as a single dose on day 11. A single 500 ug dose of carboprost was given 48 hr after the commencement of the FSH-P / PMSG. All the cows were inseminated 3 times at 12 hr interval with liquid semen starting from standing estrus. The number of CL and unovulatory follicles was 8.33±1.51 and 1.16±0.52 in FSH-P group and 12.8±2.15 and 5.60±0.10 in PMSG group, respectively.

——x——x——x——

The gonadotrophic substance in PMSG was first described in 1930 (Cole and Hart, 1930). Later it was shown to have both LH and FSH like property. PMSG has been extensively used as a stimulator to follicle development but presence of PMSG in blood circulation for a long time might have an adverse effect on quality of developing embryos. Hence, follicle stimulating hormone of pituitary origin was used commonly to superovulate cows (Totey *et al.* (1991)

An investigation to produce limited multiple ovulation with PMSG (Moore, 1975) or FSH (Wildt *et al.* (1975) led to the conclusion that the methods used were extremely variable and unreliable. Some workers have reported satisfactory and predictable response of FSH compared to PMSG (Monniaux *et al.* 1983) while Slimane and Quali (1990) found no difference between the superovulatory response (in terms of number of ova / embryos) of two gonadotrophins whereas Yadav *et al.* (1986) reported better response with PMSG than FSH-P.

MATERIALS AND METHODS

Eleven crossbred animals (Hostein Friesian, Jersey, Brown Swiss, Hariana) showing regular cyclic activity with active corpus luteum (CL) and no signs of ovarian or uterine pathology were selected and randomly divided into two superovulatory treatment groups. Group I (6 cows) was superovulated on day 11 of estrous cycle and received 40 mg of FSH-P (Schering Corporation, USA) in decreasing dose schedule, twice daily for 4 days (7/7, 6/6, 5/5, 2/2.) Group 2 (5 cows) was treated with 2500 IU Folligon (PMSG, Intervet, Holland) I / M on day 1 of estrous cycle. A day before treatment, the cows of both groups were palpated per rectally for the presence of CL and activity of ovaries. The superovulatory estrous in both groups were induced by injecting 500 ug of prostaglandin (PG) Carboprost tromethamine, Upjohn Ltd., Suxxes, UK) through I / M route, 48 hrs after commencement of FSH / PMSG treatment. The

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animals were closely observed for estrus by parading vasectomised bull in the byre at 6 hr interval and studied for duration of estrus, symptoms of estrus and intensity of estrus as per Singh and Kharche (1985). The superovulatory response were assessed by number of CL and unovulatory follicles, palpated per rectum and animals were flushed non-surgically on day 7 of estrus. Modified DPBS supplemented with 0.1% BSA was used as flushing medium. After flushing, cows in both the groups were treated with 500 ug of PG and studied for commencement of estrus and length of estrous cycle.

RESULTS AND DISCUSSION

Percentage of cows responded was more with PMSG (100%) as compared to FSH-P (83.33%). The average response to superovulation as determined by the number of CL palpated rectally on the day of flushing was more with PMSG (12.8±2.15) than with FSH-P (8.33±1.51). Yadav *et al.* (1986) reported significantly higher ovulation rate with PMSG than that of FSH-P in lactating cows. The superovulatory response in PMSG treatment observed in the present study was slightly better than Mishra *et al.* (1992) and also with FSH-P treatment was slightly better than Totey *et al.* (1991) and Agarwal *et al.* (1993). The ovulation rate between the two ovaries did not differ significantly but it was more in right ovary than that of left ovary.

More unovulated follicles (8 cm) were found with PMSG (5.6±0.50) than with FSH-P (1.16±0.52) because of longer half life of PMSG (Monniaux *et al.* 1983) thereby stimulating the folliculogenesis. Thus, these follicles presumably stimulatd to grow but failed to ovulate because of incomplete maturation of these follicles at the previous LH surge. Secondly, the higher progesterone on the day of superovulatory estrus soon after LH surge inhibits the ovulation. However, GnRH injection at the standing estrus solved this problem (Agarwal *et al.* 1993).

Following PG injection, PMSG treated cows expressed the estrus symptoms much earlier than FSH-P treated cows (34.80±6.68 hr Vs. 46.18±4.81 hr) probably due to early accelerated production of estradiol after gonadotrophin treatment. These findings are in agreement with Yadav *et al.* (1986) These data support the possibility of a relationship between the length of interval from PG to estrus and ovulation rate. An increasing interval from PG injection to observed estrus coincide with a decrease in ovulation rate (24 to 48 hr - 12.37±1.13 CL Vs. 60 to 72 hr - 5.50±1.52 CL). The pronounced estrus symptoms were observed on those cows which come to estrus within 48 hr of PG treatment.

Embryo recovery was poor only one ova was recovered in FSH-P treated group where as seven embryos were recovered from PMSG treated group. Out of seven embryos, one 2-cell, three 4-cell and three were compact morula. Non-recovery of embryos probably due to lack of egg uptake by the infundibulum due to intense ovarian reaction (Becker and Pinheiro, 1986). Premature or delayed ovulation or delayed or accelerated embryo descent into estrus due to hormonal imbalance (Harper and Chang, 1971; Becker and Pinhero, 1986) or even resulted in expulsion of vagina (Booth *et al.* (1975)

Post flushing PG injection brought the cows into estrus between 3 to 10 days. These observations are in agreement with Lopez-Barbella *et al.* (1977) and Rowe *et al.* (1978). The subsequent estrous cycle length after post flushing PG injection was shorter in PMSG treated group (12.40±2.27 days) than in FSH-P treated group (18.40±0.50 days).

REFERENCES

Agarwal, S.K., Taneja, V.K., Yadav, M.C., Sanwal, P.C. and Shankar, U. (1993). Effect of gonadotrophin releasinghormone on superovulation response, embryo recovery and their quality in crossbred cattle in the tropics. Indian J. Anim. Sci. *63(2):*131-136.

Becker, W.A. and Pinheiro, L.E. (1986). Ovarian response to superovulation in Nellore cows (*B. taurus, B. indicus).* Theriogenology *25:*785-793.

Booth, W.D., Newcomb, R., Strange, H., Rowson, L.E.A. and Sacher, H.B. (1975). Plasma estrogen and progesterone inrelation to superovulationand egg recovery in the cow. Veterinary Record *97:*366-369.

Cole, H.H. and Hart, G.H. (1930). The potency of blood serum of mares in progressive stage of pregnancy in effecting the sexual matu;rity of the immature rat. Am. J. Physiol. *93:*57-68.

Harper, M.J.K. and Chang, M.D. (1971). Some aspects of mammalian eggs and spermatozoa. Advances in Reproductive Physiology, Vol.5, pp. 167 (Ed) Bishop M.W.H., Logos Press Ltd., London, UK.

Lopez-Barbella, S.R. WArwick, A.c., Wise, T.H., Hardin, D.R., Thimpson, R.J. and Fields, M.J. (1977). $PGF_2$3/4 regression of multiple CL in bred vs. cycing cows. J. Anim. Sci.*45:*133.

Mishra, A.K., Joshi, B.V. and Nair, H.K. (1992). Preliminary trials of multiple ovulation and embryo transfer in cows under field coditions. Indian J. Anim. Reprod. *13(1):*16-17.

Moniaux, D., Chupin, D. and Saumande, J. (1983). Superovulatory responses of cattle. Theriogenology, *19(1):*55-81.

Moore, N.W. (1975). The control of time of estrus and ovulation and the induction of suprovulation inthe cattle. Aust. J. Agric. Res. *26:*295-304.

Rowe, R.F., Delcampl, M.R. and Ginther, O.J. (1978). Effect of PGF_2 on return of estrus onsuperovulated cows. J. Anim. Sci. *47(Suppl. 1:*) 387.

Singh, M.M. and Kharche, K.G. (1985). Sexual behaviour and reproductive efficiency of crossbred cows. Livestock Adviser, *10(4):*9-13.

Slimane, N. and Quali, F. (1990). embryo transfer in cattle in Tunisia. In: Amelioration genetique de bovine climate stud mediterranen, Wageningen, Netherlands, Pudoc (1990). 189-195 (cf ABA 1991,Vol.59, No.5).

Totey, S.M., Gurprit Singh, Taneja, M., Pawshe, C.H., Gurcharan Singh and Chillar, R.S. (1991). Nonsurgical embryo transfer in crossbred and nondescript cows. Indian J. Anim. Reprod.*312(1):*42-46.

Wildt, D.E., Woody, H.d. and Dekelow, W.R. (1975). Induction of multiple ovulation in the cow with single injection of FSH and HCG. J. Reprod. Fet. *44:*583-586.

Yadav, MC., Walton, J.S. and Leslie, K.E. (1986). Plasma concentration of LH and progesterone during superovulation of dairy cows using FSH or PMSG. Theriogenology *26:*524-540.

IJAR. 16(1), 1995; 15-17

Study On Certain Factor(s) In Pregnant Buffalo Uterus That Suppress Compensatory ovarian Hypertrophy in Mice

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ABSTRACT

The compensatory ovarian hypertrophy supressing activity in 20% ammonium sulphate precipitates from 0.9% saline extracts of maternal caruncles and inter caruncular uterine tissue from pregnant uterus was examined. The extracts were injected into mice on the morning of dioestrus after hemispaying, while the control mice received bovine serum albumin. The mice were sacrificed after 72 hours and the ovaries were weighed. The degree of COH was expressed as percent increase in weight of right ovary to that of the left. COH supressing activity was found to be present in inter caruncular uterine tissue, but absent in maternal caruncles. This inhibitory factor(s) is inactivated by trypsin digestion and may play a role in regulation of follicular dynamics during pregnancy.

——x——x——x——x——

The suppression of follicular development and ovulation during pregnancy is brought about by the higher levels of progesterone, which suppresses circulatory-levels of FSH & LH (Rexroad and Casida, 1975). In addition, possibility of secretion of some factors from endometrium having important role in regulating follicular dynamics during pregnancy can not be ruled out. Recently a factor from rat uterine eithelium has been detected, which has an inhibitory activity on prolaction secretion from pituitary (Gorospe and Freeman, 1985). It could, therefore, be possible that in suppression of follicular growth and prevention of ovulation, endometrium may play certain role by secreting some agents that may act on pituitary through suppression of release of FSH in a manner similar to that of inhibin. The present experiment was designed to test this hypothesis using maternal caruncles and inter-caruncular uterine area from prgnant buffaloes as test materials.

MATERIALS AND METHODS

Pregnant buffalo utri of 2-4 months gestation, were obtained from abattoir, within an hour of slaughter, and transported to laboratory in chilled (4°C) 0.9% saline. Gestation age was estimated by measuring crownrump length of the foetuses as per Singh *et al.* (1963). Foetal cotyledons were carefully seperated from maternal caruncles. Pools of maternal caruncles and inter-caruncular uterine endometrium (80-100g) were collected and homogenized in three volumes of chilled 0.9% saline in a similar manner, seperately. The homogenates were centrifuged at 4000 rpm for 30 min at room temperature (about 20°C). The supernatants were treated with 20% (W/V) ammonium sulphate, and kept in refrigerator overnight to ensure complete precipitation of proteins. Next morning, the precipitates were collected by centrifugation at 4000 rpm for 30 min at room temperature suspended in distilled water and dialyzed for 96 h against distilled water at 4°C. After dialysis the material was used for bioassay. Control group of mice received bovine serum-albumin. Estimation of protein in different samples was done by the method of Lowry *et al.* (1951).

Young adult female mice of swiss albino strain aged 35-40 days, were kept under a

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normal day light schedule with free access to feed and water. The mice were ovariectomized unilaterally under ether anesthesia between 10 and 12 hour. The left ovaries were removed and weighed immediately.

Ammonium sulphate fractions of maternal caruncular and inter caruncular uterine tissue extracts, trypsin digested (50 ug trypsin / mg protein incubated for 2 h at 37°C in Tris-EDTA buffer, pH 7.4) inter caruncular uterine tissue extracts were injected subcutaneously in the experimental mice while control mice were injected with bovine serum albumin. Each mouse received 200 ug of protein in 0.5 ml volume. Seventy two hours later, all the mice were killed and their right ovaries removed and weighed. The degree of COH for each mouse was calculated as the percent increase in the weight of right ovary (at sacrifice) compared with that of left ovary (at hemispaying). Student's 't' test was used to analyse the data.

RESULTS AND DISCUSSION

Table 1 showed that the hypertrophy of right ovary was significantly ($P<0.05$) suppressed in the mice injected with 200 mg of protein from intercaruncular uterine tissue extract as compaired to controls (36.±2.95 vs 60.8±5.90%). The maternal caruncular tissue extract did not show such COH suppresing activity. Trypsin digestion of inter caruncular uterine tissue extract abolished its COH suppressing activity.

Supression of COH in mice was observed on administration of 200 ug protein from intercaruncular uterine tissue extract, indicating that intercaruncular uterine tissue contained certain inhibitor(s). This was reported in buffalo foetal cotyledons (Maurya *et al.* 1990). Surprisingly such COH suppressing activity was found absent in protein precipitate obtained from maternal caruncular extract. This inhibitory activity was lost on trypsin digestion conforming its proteinaceous nature. These results indicated that like the foetal cotyledonary extract the inter-caruncular uterine tissue extract also possessed inhinbi-line activity and was proteinaceous in nature. It was difficult to give satisfactory explanation for clear cut variation between the activity of inter-caruncular area and maternal carcunles. Although the ruminant placenta was epitheliochorial (Jainudeen and Hafez, 1980), in the buffalo placenta the areas around crypts in maternal caruncules showed considerable denudation of surface epithelium presumably due to massive effect of foetal cotyledonary villi (Raja Ram, 1982). It remains to be seen if this denudation of endometrial epithelium of caruncular region during pregnancy accounts for this loss of COH suppressing activity in maternal caruncles. The mechanism of action of the inhibitor detected in inter-caruncular area is not yet known. It may be suggested that this could be in the same way as inhibin acts, since compensatory mechanism following unilateral ovariectomy in the cyclic rat involves increased FSH secretion (Benson *et al.* (1969). This inhibitor(s) could play an important role in endocrine interactions involved in foeto-maternal relationship and thereby regulating follicular dynamics during pregnancy in the bovin.

Table 1: Effect of Ammonium sulphate precipitate of intercaruncular uterine tissue extract (with and without trypsin digestion) and maternal caruncular extract on compensatory ovarian hypertrophy in mice (Mean±S.E.M.)

Treatment	No. of Mice Treated	Weight of Mice (g)	Weight of left ovary (mg)	Weight of right ovary (mg)	% Hypertrophy of right ovary
Bovine serum albumin	6	25.5.±0.99	2.28±0.17	3.64±0.49	60.8±5.90
Inter caruncular uterine tissue	6	23.7±1.06	2.05±0.14	2.78±0.17	36.4±2.95*
Maternal caruncles	6	29.2±0.66	2.83±0.23	4.55±0.38	62.0±5.99
Trypsin digested Intercaruncular Uterine tissue	6	23.7±1.12	2.04±0.21	3.23±0.33	58.6±2.32

* Significantly different from control value ($P<0.05$)

REFERENCES

Benson, B; Sorrentino, S. and Evans, J.S; (1969). Increase in serum FSH following unilateral ovariectomy in the rat. Endocrinology, *84:* 369-374.

Goropse, W.C. and Freeman, M.E. (1985). Detection of prolactin inhibitory activity in uterine epithelial cell secretions and rat serum. Endocrinol. *116:* 1159-1564

Jainudeen, M.R. and Hafez, E.S.E. (1980). Gestation, prenatal physiology and parturition. In : E.S.E. Hafez (ed.) 4th ed. pp. 247-283, Lea and Febiger, Philadelphia.

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

Maurya, R.B. and Pant, H.C. (1990). Buffalo fetal cotyledons containcertain factors that suppress compensatory ovarian hypertrophy in mice. Anim. Reprod. Sci; *22:* 1-7.

Raja Ram (1982). Gross, histological and histochemical observations on the placenta of buffalo. PH.D. Thesis submitted to C.S.A. University of Agriculture and Technology, Kanpur.

Rexroad, C.E; Jr. and Carida, L.E; (1975). Ovarian follicular development in cows, sows, and ewes in different stages of pregnancy as effected by number of corpora lutea in thesame ovary. J. Anim. Sci; *41:* 1090-1097

Singh, S; Sanger, O.P.S. and Singh, S.N; 1963. Placental development of buffalo, Bos bubalis L. Agra Univ J. Res. *12:* 197-245.

IJAR. 16(1), 1995; 18-21

Changes in Electrolytes of Antral Follicles in Goat

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ABSTRACT

Biochemical analysis of six categories of normal developing antral follicles of goat (Capra hircus) was conducted to study the sequential pattern of changes in electrolytes. The quantity of (Na) decreased from 5281.63±1494.53 in category I (early antral) folices to 4065.53±781.37 ug/g wet weight, in category V (preovulatory) follicles. A corresponding increase in K content was observed from 141.54±56.63 in category I to 211.87±26.16 ug/g in category VI (ovulatory) follicles. The variations observed in electrolytes have been discussed in relation to homeostatic dynamism operating in follicles at different stages of development and maturation.

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Studies on follicular growth and maturation in various mammalian specis have demonstrated the changes in cellular complexity and in the synthesis, accumulation and transport of different biomolecules as the growth progress (Zamboni, 1974; Sangha and Guraya, 1889a, b; Sharma and Guraya, 1990). Most of the earlier studies were confined only to the histochemical demonstration of various types of macromolecules like lipids, proteins, carbohydrates and enzymes (Guraya, 1974, 1985; Sharma and Guraya 1992). Although a few studies have been carried out to investigate biochemical changes in macromolecules vis-a-vis follicle growth in rodents (Sangha and Guraya 1989a, 1989b) yet very limited information is available on domestic animals (Hafez, 1987). Keeping in view the existing lacunae in literature present investigation was conducted to study the pattern of changes in Na and K will be very useful to determine their role in ovarian function and thus fertility, as investigated here for the goat. The details of biochemical and physiological interrelationships will provide an index to examine deviation insituation wherein normal growth and development of antral follicles is threatened in vitro system.

MATERIALS AND METHODS

During the present investigation, the goat (Capra hircus) ovaries were obtained from the abattoirs of Karnal and Chandigarth. The material were brought to the laboratory at 0°C. The ovarian follicles at different stages of development and maturation were manually separated with the help of fine forceps and needles under the dissecting microscope. These follicles were then categorized into six categories on morphometric bases.

Extraction Techniques: Each category of the manually separated follicles was washed first with deionized water. Thereafter, the weight and number of follicles were recorded. At the minimum each sample was weighing not less than 200 mg. The samples were digested in long necked roundbottom flasks with triple acid (conc. nitric acid : 70% perchloric acid : conc sulphuric acid, 10:3:1) in the ratio of 1:10 (W/V). This process was executed by heating the contents till most of the triple acid mixture evaporated from the flask. The contents of each flask were then washed with 2 ml of deionized water and were stored in plastic vials at 4°C.

The concentration of trace elements was determined by atomic absorption spectophotometer (Ludmilla, 1976) installed at Central Soil Salinity Research Institute, Karnal (Haryana). Students 't' test was used to test the validity of results (Zar, 1984).

RESULTS

1. Sodium (Na)

The quantity of Na increased from 105.84 mg / follicle in category I to 1836.13 mg / follicle incategory V. This increase was steady upto pre-ovulatory follicle (V). The variations observed were statistically non-significant between the adjacent categories. But differences were statistically significant between category V and I, II and III, IV. However, Na amount increased dramatically from category V onwards (Table 1) The variations in Na content on ug / g of tissue basis showed a negative trend. The maximum value observed was in the follicles of category I (5281.63) and a minimum was observed in category V (4065.53). However, a slight increase in Na content was observed in ovulatory follicles. All these variations were statistically non-significant.

2. Potassium (K)

The amount of K on mg / follicle basis increased gradually from 2.97 in follicles of category I to 172.32 in ovulatory follicles (VI) (Table 1). The amount of K present in category VI was significantly different from category I, II and III, whereas differences between category I, III and V were statistically significant. On ug / g of tissue basis only minute variations observed. These changes were not significant.

DISCUSSION

The data observed in the present study revealed that concentration of Na declined, whereas concentrations of K increased, with the advancement in follicular size. The values observed in the present finding are in agreement with the earlier findings (Knudson *et al.* (1979) Chang *et al.* (1976) David *et al.* (1973) and Guraya, 1985). The higher values of Na and K observed in the present investigation are in accordance with the observation of Knudsen *et al.* (1979) , wherein they have reported slightly higher value of K from the follicular fluid of small and medium-sized follicles which reflect a physiological process of follicular maturation Previous investigators (Schuetz and Anisowicz, 1974; Chang *et al.* (1976) have reported even higher K values in porcine follicular fluid from ovaries obtained from slaughter house. The anoxia and intra-follicular acidosis (knudsen *et al.* (1978) result from ischaemia before collection of follicular fluid could lead to post-mortem degeneration of granulosa cells with the resultant loss of intra-cellular K into the follicular fluid. The Na content observed in present investigation varied from 406 to 528 mg / 100 ml in contrast to 330 mg / 100 ml of Na present in the blood plasma (Knudsen *et al.* (1979). The K levels were also approximately 2 to 2.5 times higher than that of blood plasma levels. These higher levels are indicative of active metabolic status of the granulosa cells of the developing follicles. Keele and Neil (1964) have clearly mentioned that when isolated slices of renal cortex or brain are placed in a medium of suitable composition at 37°C and in the presence of oxygen and a food stuff substrate (1—glutamate for brain and — ketoglutrate for kidney), the cells actively take up the substrate until its intracellular concentration may be as high as 2.5 times that of outside, although at the same time there is rapid oxidation of substrate. The combined concentration of intra-cellular K and Na may exceed 250 mEq. per litre as compared with only 155 mEq. per litre in the extra cellular fluid. It seems, therefore, certain that the intracellular osmotic pressure greatly exceed that of plasma and is maintained by the active forces. It is, therefore, logical to deduce that the decline in the concentration of Na with the advancement in the follicle size is a direct reflection of the number of granulosa cells present, as the category I follicle (early antral follicle) advances to category VI (ovulatory) follicle, a tremendous enlargement of the follicle dimension is largely due to the accumulation of antral fluids. Corresponding to this enhancement in size, the mitotic index of granulosa cells do not keep pace with this increased size, with the result that the number of granulosa cells per unit volume, falls in the ovulatory follicles. Since follicular fluid is an exadute of blood plasma, modified by

granulosa cells (David *et al.* 1973). The slight decline in large Na concentration of follicles that we have reported in the present investigation is because of the difference in the number of granulosa cell bathed in the follicular fluid. This cumulative factor (granulosa cell Na, and follicular fluid Na content) accounts for the decline observed in Na content. The rising titre of K observed during folliculogenesis is possibly due to the extraction of intra-cellular K. This is in agreement with the earlier findings of Knudson *et al.* (1978); Chang *et al.* (1976) where in they have clearly stated that higher K content observed in the material had from slaughter houses is largely due to anoxia and interafollicular acidosis. The present observations do not support the earlier findings of Schuetz and Anisowicz (1974) wherein they have demonstrated an inverse relation between follicle size and follicular K level, in slaughter house material and have assigned the decrease to greater surface area volume ratio. The slightly elevated levels of K corresponds positively to declining Na content observed during the present investigation for the simple homeostatic dynamism (Guraya, 1985). The preovulatory rabbit follicles increases in volume at an average rate of 0.15 ul / h (Rondell, 1964). This increase in volume represents the movement of water from the capillary bed in the follicle wall to the antrum, drive, by hydrostatic and / or osmotic pressure gradients.

Acknowledgement: Authors are thankful to Director C.S.S.R.I, Karnal and Chairman Department of Zoology Kurukshetra University, Kurukshetra for providing necessary facilities. University scholarship rendered to Rajeev Vats by Kurukshetra University, Kurukshetra is acknowledged.

Table 1 Variations in the electrolytes, Na and K (mg / folicle) in different categories of follicles.

(Figures in in parnthesis represents Hg / g wet weight of tissue)

Catagory	*State of follide*	*Size (mm)*	*Na*	*K*
I	*Early antral*	*<2*	*105.84**±40.55*	*2.97**±0.17*
			(5281.63±1494.53)	*(141.54±56.63)*
II	*Small antral*	*2—3*	*369.24**±63.11*	*17.06**±4.59*
			(4775.37±1294.36)	*(164.45±8.72)*
III	*Medium antral*	*3—4*	*657.76**±87.78*	*22.55**±4.65*
			(4740.16±1713.86)	*(164.66±7.01)*
IV	*Large antral*	*4—5*	*1780.94**±578.07*	*66.84±17.67*
			(4613.76±1205.50)	*(164.53±73.16)*
V	*Preovulatory*	*5—6*	*1836.13**±577.42*	*79.34**±33.67*
			(4065.53±781.37)	*(173.58±53.98)*
VI	*Ovulatory*	*>6*	*3942.85**±1039.49*	*172.32**±42.46*
			(4476.09±2125.75)	*(211.87±26.16)*

**P<0.05 (t-test)*

REFERENCES

Chang, S.C.S., Jones, J.D., Ellefson, R.D. and Ryan, R.J., 1976. The porcine ovarian follicle : 1 Selected chemical analysis of follicular fluid at different developmental stages. Biol. Reprod., 15: 321-328.

David, A., Frenkel, G., and Kraicer, P.F. 1973. Chemical composition of Rabbit follicular fluid. Fertility and sterility., *24 (3):* 227-229.

Guraya, S.S. 1974. Morphology, histochemistry and biochemistry of human orgenesis and ovulation. Int. Rev. Cytol., *37:* 121-147.

Guraya, S.S. 1985. Biology of ovarian follicles in mammals. Springer Verlag, Berlin, Heidelberg, New York.

Guraya, S.S., Parminder Kaur, Sharma, R.K. 1991 Histochemical and Biochemical Studies on Esterase Activity in the Rat Ovary *29 (3):* 161-172.

Hafez, E.S.E. 1987. Reproduction in Farm Animals. Ed. 5th., Lea and Febiger, 600 Washington Square Philadelphia. U.S.A.

Keele, C.A. and Neil, E. 1964. Samson wright's Applied Physiology Ed. 11th, Oxford Medical Publication.

Knudsen, J.F., Litkawski, L.J., Ulitson, T.L., Guthrie, H.D. and Batta, S.K. 1979. Follicular fluid electrolytes and osmolality in cyclic pigs. J. Reprod. Fert., *57:* 419-422.

Knudsen, J.F., Litkowski, L.J., Ulitson. T.L., Guthrie, H.d. and Batta, S.K. 1978. Concentrations of hydrogen ions, oxygen, carbon dioxide and bicarbonate in porcine follicular fluid. J. Endocr., *79:* 249-250.

Ludmilla, D. 1976. Chemical analysis by Atomic Absorption Spectroscopy. Varian Tecthran Pvt. Ltd., Melbourre, Australia.

Rondell, P. 1964. Follicular pressure and distensibility in ovulation. Am. J. Physiol., *207:* 509-594.

Sangha, G.K. and Guraya, S.S. 1989a. Biochemical changes in lipids during follicular growth and corpora lutes formation and regression inrat ovary. J. of Experimental Biology. *27:* 998-1000.

Sangha, G.K. and Guraya, S.S. 1989b. DNA, RNA and Protein changes in Rat Ovarian follicles Proc. Indian Matn. Sci. Acad. B55 No. *3:* pp. 155-158.

Schuetz, A.W. and Ansowicz, A. 1974 Cation and protein composition of ovarian follicular fluid of the pig : relation to follicle size. Biol. Reprod., *11:* 64-72.

Sharma, R.K. and Guraya, S.S. 1990. Granulosa heterogenity : A histochemical, lectin staining and scanning electron Microscopic study of Rattus rattus ovary. Acta Embryol. Morphol. Exper. n.s., *11 (2):* 107-121.

Sharma, R.K. and Guraya, S.S. 1992. Lectin staining studies on follicular atresia in House rat RAttus rattus.Acta. Morphol. Hung. *40 (1-4):* 25-34.

Zamboni, L. 1974. Fine morphology of the wall and follicle ceil oocyte association. Reprod.. *10:* 125-149.

Zar, J.H. 1984. Biostatistical Analysis. Prentice-Hall. Inc., Englewood Cliffs. N.J.

IJAR. 16(1), 1995; 22-24

Haemoglobin Polymorphism, Transferrin Polymorphism and Sex Chromatin (Drumstick) frequency in Normal Cycling and Delayed Pubertal Crossbred Heifers*.

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ABSTRACT

Haemoglobin Polymorphism, transferrin polymorphism and sex chromatin (drumstick) frequency were studied in normal cycling and delayed pubertal crossbred heifers. Three haemoglobin types (AA, AB and BB) were demonstrated in the crossbred heifers. The gene frequency of A and B genes were 0.50 and 0.50 innormal cycling and 0.30 and 0.70 in delayed pubertal crossbred heifers. Five transferrin types (DN, DK, NN, NK and KK) were demonstrated in crossbred heifers. The KK type transferrin was found in greater number of normal cycling (33.33 per cent) and delayed pubertal (33.33 per cent), whereas, NK type was found in less number of normal cylcing (16.66 per cent) and delayed pubertal (8.33 per cent) crossbred heifers. Out of 36 delayed pubertal heifers 15 (41.66 per cent) had zero drumstick frequency, whereas, out of 12 normal cycling heifers none (0.00 per cent) had zero drumstic frequency.

——x——x——x——

Besides increasing milk production, one of the important objectives of crossbreeding indigenous cattle was to reduce the age of puberty / maturity to harvest its desired advantages. However, the success achieved in this aspect seems to be questionable. The recent reports suggest that the age of puberty in crossbreds is showing increasing trend (Baghel, 1988; Deshmukh and Kaikini, 1989). Velhankar *et al.* (1977) reported that 37 heifers with Hb A expressed puberty and sexual matu;rity in 857.00±24.34 and 905.15±30.21 days, respectively. Whereas, animals with Hb AB required 899.23±28.40 and 954.76±28.71 days, for puberty and sexual maturity, respectively. Singh and choudhary (1989) found significant ($P<0.01$) effect of transferrin on age at first estrus, whereas other traits were found non significant. The mean age at first estrus was lowest (647.17 days) in crossbred cows with Tf EE. The present investigation was undertaken to study the haemoglobin polymorphism, transferrin polymorphism and sex chromatin (drumstic) frequency in normal cycling and delayed pubertal crossbred heifers.

MATERIALS AND METHODS

For haemoglobin typing, aporoximately 5 ml blood was collected by venepuncture of the jugular vein in a sterile vial containing 0.5 ml 4 per cent sodium citrate. Haemolysate was prepared as per the procedure described by Verma and Rawat (1980).

Just after the collection of blood, the sterilized test tube was kept in slanting position and blood was allowed to coagulate for 1 to 1.30 hrs. Then the sample was centrifuged at 3000 rpm for 15 minutes. The serum was applied in the form of streak with sample applicator. Standard

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paper electrophoresis was adopted to identify and classify haemoglobin and screening of sexchromatin was done as per the method described by Bhatia *et al.* (1982).

RESULTS AND DISCUSSION

Haemoglobin Polymorphism:

The gene frequency of A and B genes were 0.50 and 0.50 in normal cycling and 0.30 and 0.70 indelayed pubertal crossbred heifers (Table 1).

The lower frequency of A type gene might be responsible for delayed puberty. These findings are similar with those of Velhankar *et al.* (1977), who reported that heifers with Hb AA type expressed puberty at an early age than heifers with Hb AB type.

Transferrin Polymorphism:

The transferrin types were identified on the basis of their migration rate and grouped accordingly and subsequent genotypic frequencies were calculated and presented in Table 2.

The KK transferring was found in more number (33.33 per cent of heifers) and NK type in less number of normal cycling 16.66 percent) and delayed pubertal (8.33 per cent) crossbred heifers indicating that possibly there is no role of transferrin type in attainment of puberty. However, Singh and Chouldary (1989) observed lowest age at first estrus in cows with EE tranferring type.

Five types of transferrin (DN, DK, NN, NK and KK) were recorded in the present study, whereas, six types were recorded by Singh *et al.* (1972) in Hariana, Ongole, Gir and Kankrej cows and Singh and Choudhary (1987) in crossbred cows which might be due to breed differences.

Sex Chromation (drumstick) frequency:

Out of 36 delayed pubertal heifers 15 (41.66 per cent) had 0 drumstick frequency,whereas, out of 12 normal cycling heifers none (0.00 per cent) had 0 drumstick frequency (Table 3). This clearly indicate that absence of drumstick may be the diagnostic feature of the genetic cause of delayed puberty in crossbred heifers. The present findings agree with those of Bhatia and Shanker (1982) who reported low frequency of sex chromatin appendages in the culled females exhibiting disturbed fertility as compared to normal females.

Table 1 : Gene frequency for Hb types in normal cycling and delayed pubertal crossbred heifers.

Group of heifers	No. of heifers	Observed Hb types			Gene frequency	
		I (AA)	II (AB	III (BB)	A	B
Normal	12	4	4	4	0.50	0.50
Delayed pubertal	36	5	12	19	0.30	0.70

Table 2. Trnasferring types in normal cycling and delayed pubertal crossbred heifers.

Group of heifers	No. of heifers	Transferring types				
		DN	DK	NN	NK	KK
Normal cycling	12	—	3	3	2	4
Delayed pubertal	36	5	6	10	3	12
Normal cycling (%)	—	—	25.00	25.00	16.66	33.33
Delayed Pubertal (%)	—	13.88	16.66	27.77	8.33	33.33

Table 3: Drumstick frequency in normal cycling and delayed pubertal crossbred heifers.

Group of heifers	No. of heifers	Drumstic frequency		
		0	1	2
Normal cycling	12	—	11	1
Delayed pubertal	36	15	20	1

REFERENCES

Baghel, S.L. (1988). Incidence associated causes andestrus induction indelayed pubertal crossbred heifers. M.V.Sc. & A.H. Thesis, J.N.K.V.V., Jabalpur.

Bhatia, S. and Shanker, V. (1982). Practical application of sex chromatin studies in the investigation of disturbed fertility in female cattle. Proc. IInd Wod. Congress on Genetics applied to Livestock Production. 4-8th Oct. Madrid, Spain Sym., *I(3):*483-488.

Bhatia, S., Shanker, V. and Mishra, R.R. (1982). Sex chromatin studies. Wld. Revies of Anim. Prod. *XVIII(3):*65-70.

Deshmukh, A.W. and Kaikini, A.S. (1989). Age at maturity in Sahiwal crossbred heifers with three levels of inheritance, IJAR. *10(1):*1-3.

Singh, Ashok and Choudhary, R.P. (1987). Serum transferring polymorophism in Zebu and crossbred cattle. Indian Vet. J. *64:*927-930.

Singh, Ashok and Choudhary, R.P. (1989). Serum transferrin polymorphism and its association with growth and reproductive traits in Sahiwal and Crossbred. Indian Vet. J. *66:*933-937.

Singh, H., Batabyal, A.K., Bhatia, S.S. and Khanna, N.D. (1972). Serum transferrin polymorphism in cattle breeds. A note Indian J. Anim. Sci. *42(9):*650-652.

Velhankar, D.P., Tadpatrikar, N.S. and Sane, C.R. (1977). Haemoglobin polymorphism in relation to puberty and sexual maturity in Gir heifers. Indian J. Dairy Sci. *30(2):*134-137.

Verma, D.N. and Rawat, J.S. (1980). Electrophoretic pattern of haemoglobin components in the blood of various strains of White Leghorn, Poultry Adviser, *13:*10-11.

IJAR. 16(1), 1995; 25-27

Variations in the Plasma Levels of Copper and Zinc in Crossbred Cows with early and Delayed Post-partum Conception.

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ABSTRACT

The plasma levels of copper and zinc were measured at monthly intervals by atomic absorption spectrophotometer in postparturient cows conceiving within 120 days post-partum (Group-I) and not-conceving within 120 days post-partum (Group-II). The plasma levels of both copper and zinc were found significantly higher ($P<0.01$) in cows of Group-I (Average 132.65±6.46 and 172.30±9.24 µg / dl, respectively) compared to cows of Group-II (Average 102.34±2.60 and 105.32±6.59 µg / dl, respectively) indicating that low levels of both copper and zinc may be related to delayed post-partum conception

——x——x——x——

Delayed conception in cattle causes enormous losses to cattle owners. About 25% of the dairy cows are culled for reproductive reasons (Young, *et al.* 1983). Many factors like uterine pathology, debilitating diseases, poor herd management, poor semen and also poor nutrition including minerals may be implicated in causing delayed conception (Chauhan and Kesy, 1984). It is possible that delayed conception in apparently healthy cows may be due to deficiencies of trace minerals and their blood levels may be of potential aid in characterizing the problem of delayed conception. The present study was proposed to estimate plasma levels of copper and zinc incows conceiving and not-conceiving within 120 days post-partum.

MATERIALS AND METHODS

The study was conducted on 14 post-parturient cows of second lactation and of group 25% Sahiwal, 25% Jersey and 50% Red-Dane (SXJXRD) and belonging to the Livestock Research Centre of G.H.Pant University of Agriculture and Technology, Pantnagar. These cows had normal breeding history and had no apparent reproductive abnormality. The experimental cows remained in a group of 40 cows having milk yield 10-12 lit / day / cow throughout the period of the study and both the fodder and the concentrate rationwere supplied also in group. All the animals of the herd were closely observed for the behavioural symptoms of oestrus regularly, twice daily. These cows were bred artificially (but not before 60 days post-partum). The pregnancy was confirmed by the per-rectal examination. The cows under study were grouped as follows:

Group I : 4 cows conceiving within 120 days post-partum.

Group II : 10 cows not-conceiving within 120 days post-partum.

About 10.0 ml blood was collected at day 0, 30, 60, 90 and 120 post-partum from each cow in clean and sterilized tubes containing 200 ul of 10% EDTA in normal saline. Plasma was stored at - 20°C till estimation of copper and zinc levels in diluted samples (dilution rate 1: 25) using atomic absorption spectrophotometer.

RESULTS AND DISCUSSION

The mean levels of both copper and zinc in different groups of cows are presented in Table-1. The results indicated that the plasma levels of these trace minerals were always higher incows of group-I compared to group-II and the differences were statistically significant except for copper at day 0 and for zinc at day 0 and 30.

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The differences in the plasma levels of these trace minerals in the two groups of cows may be attributed to the differences in the body reserves, oral intake and excretion thorough different excretory channels. The higher levels of both copper and zinc in cows of group-I in comparison to cows of group-II indicate that the plasma levels of these trace minerals may be of importance in relation to postpartum conception. The present findings are in agreement with Kappel *et al.* (1984). Poole *et al.* (1986), Potte *et al.* (1988), Singhal and Lohan (1991), Simeonov *et al.* (1989) and Fedosova *et al.* (1991) who indicated that low copper and/or zinc levels may be related to delayed post-partum conception. However, Larson, *et al.* (1980) did not find any relationship between serum copper and zinc levels and the reproductive performance of the cows and Ruksan *et al.* (1982) and Alberio, *et al.* (1985) for copper and Olieveri *et al.* (1979) for zinc with post-partum reproductive performance.

Table 1: Mean levels of copper and zinc in different groups of cows.

Days post-partum	Copper			zinc		
	Mean value (µg / dl)		t-value	Mean value (µg / dl)		t-value
	Group I	Group II		Group I	Group II	
0	126.25 ±13.34	107.50 ±8.70	1.1500S	195.00 ±4.02	140.90 ±23.27	1.1900^{NS}
30	125.00 ±16.70	97.90 ±4.18	3.1088**	166.25 ±18.52	120.00 ±13.52	1.8800^{NS}
60	123.25 ±15.93	105.30 ±4.47	2.5096**	169.50 ±27.92	107.70 ±13.65	2.2391*
90	149.50 ±17.79	102.50 ±6.57	3.7100**	171.25 ±21.63	87.50 ±10.24	3.9976**
120	139.25 ±17.16	98.50 ±5.31	3.0616**	162.00 ±9.60	70.50 ±7.51	6.777**
Over all Mean	132.65 ±6.46	102.34 ±2.60	10.3400**	172.30 ±9.24	105.32 ±6.59	4.9725**

* ($P<0.05$); ** ($P<0.01$)

REFERENCES

Alberio, R.H., Butler, H.M., Palma, C.R. and Torquott, O. (1985). Effect of temporary calf removal and/or parenteral copper on post-partum sexual activity in multiparous cows. Memorias Association Latinoamericana de Production Animals 18:144 (Anim. Breed. Abstr. *55:*3470).

Chauhan, F.S. and Kessy, B.M. (1984). Recent advances in hormonal therapy of bovine reproductive disorders. A review. Vet. Bull. *54:*991-1009.

Fedosova, N. Kh., Rassadnikov, S.A. and Kononv, G.A. (1991). The effect of Prostaglandin F_2 on ovulatory function of cows after calving. Anim. Breed. Abstr. *60:*6873.

Kappel, L.C., Ingraham, R.H., Morgan, E.B. and Babcock, D.K. (1984). Plasma copper concentration and P.C.V. and their relationship to fertility and milk production in Holstein cows. Amer. J. Vety. Res. *45:*346-350.

Larson, L.L., Mabruck, H.S. and Lowry, S.R. (1980). Relationship between early post-partum blood composition and reproductive performance in dairy cattle. J. Dairy Sci. *63:*283-289.

Olieveri, O., Valfre, F., Bosi, G. and Silvestrelli, M. (1979). Variation of Zn and Fe in blood serum of Chiana cows with differing fertility. Anim. Breed. Abstr. *50:*1941.

Poole, D.B.R., Smith, F.H., Roche, J.F. and Vaughan, R.M. (1986). Copper deficiency and bovine infertility. In Research Report, Animal Production An Foras Taluntais. Dublin, Irish Republic (Anim. Breed. Abstr. *56:*7425).

Potte, E.B., Almedia, I.L.De., Brum, P.A.R. De, Rullia, R.R, Sausa, J.C. De. and Aroeira, J.A.D.C. (1988). Reproductive performance of cattle in Piaguas in the Matto Grossoswamps. 3. Effect of mineral supplements on Variations inreproductive traits and body weight of breeding cows. Pesquisa Agropecuaria Brasileria. 23 : 87-97 (Anim. Breed. Abstr. *56:*6231).

Ruksam, B.E., Casaro, A., Jaeschke, J., Lagos, F. and Pondal, D.G. (1982). Effect of copper supplementation on fertility in heifers. Trace elements metabolism in man and animals, Berlin, German Federal Republic, Springer-Verlag, 179-182 Anim. Breed. Abstr. *51:*4258.

Simeonov, S., Petrova, L, Dremovski, G., Tsvetkova, V. and Georgiev, G. (1989). The vitamin and mineral status of blood serum inearly conceiving and high yielding cows and in those returning to service. Veterinarna Sbirka. 87 : 48-50 (Anim. Breed. Abstr. *59:*6702).

Singhal, S.P. and Lohan, I.S. (1991). Studies on the Incidence and reproductive management of infertility in breedable dairy animals in rural Hariana State. In 11th International Congress on Animal Reproduction and A.I. Univ. College, Dublin. paper No. 544. 3pp (Anim. Breed. Abstr. *59:*3841).

Young, G.B., Les, G.F., Waddington, D., Sales, D.I. and Spooner, R.I. (1983). Culling and wastage in dairy cows in East Anglia. Vet. Rec. *113:*107-111.

IJAR. 16(1), 1995; 28-31

A comparative study of Haemoglobin, Copper and Zinc concentration of post partum anoestrus cows.*

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ABSTRACT

Serum from thirty six post partum anoestrus cows and twelve normal cycling cows were collected to estimate the concentration of haemoglobin, copper and zinc. The mean serum haemoglobin, copper and zinc in post partum anoestrus cows were found to be 9.57±0.25 gm per cent, 101.31±2.74 mcg per cent and 253.87±13.43 mcg per cent as compared to 11.74±0.60 gm per cent, 114.20±6.78 mcg per cent and 314.00±17.39 mcg per cent in normal cycling animals which were found to differ significantly (P<0.05). A total of 24 post partum anoestrus animals were supplemented with trace minerals composed of copper sulphate, ferric sulphate and zinc sulphate and the rest 12 animals were left as untreated control. Eighteen out of 24 post partum anoestrus animals responded to the treatment and exhibited heat within the average period of sixty days. The test of significance for copper zinc in these animals between pre and post treatment period revealed a highly significant difference (P<0.01) but haemoglobin was found to differ significantly (P<0.05).

——x——x——x——

Ascertaining the post partum ovarian activity and associated changes are highly essential to obtain the optium fertility in cows. The probable relationship of breeding efficiency with the concentration of trace minerals post partum and the low values of haemoglobin and P.C.V. were found to be associated with anoestrus and repeaters Kumar *et al.* (1986). Therefore, the present investigation was undertaken to evaluate the different levels of certain blood constituents in post partum anoestrus animals and determine the defficiencies, if any, and their action on resumption of oestrus under local agro-climatic conditions.

MATERIALS AND METHODS

Thirty six cross-bred cows between 2½ to 9 years of age, not exhibiting oestrus for more than 90 days after parturition constituted the experimental group (anoestrus cows), whereas 12 cows exhibiting normal oestrus withn 60-90 days post partum was considered to be the control group (cyling cows). Blood serum collected from these animals was analysed for copper and zinc concentration by atomic absorption spectrophotometer as per the procedure laid down by Smith *et al.* (1979) and Dawson *et al.* (1968). The whole blood was subjected for haemoglobin by Sahli method as described by Collier (1955). The experimental animals after being clinically investigated were divided into twogroups:

Group I - Consisting of 24 animals were given the following medicaments daily for 20 days.

Copper Sulphate*	400 mg
Ferrous Sulphate**	400 mg
Zinc Sulphate***	200 mg

* Part of the M.V.Sc. thesis submitted by the first author to O.U.A.T., Bhubaneswar.

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* Copper sulfate-5-hydrate pure - E.Merck, India Ltd., Bombay.

** Ferrous sulphate (crystalline) - Qualigens Fine chemicals.

*** Zinc sulphate-7-hydrate purified - E. Merck, India Litd., Bombay.

Group II - Consisting of 12 animals were not given any treatment and kept as untreated control.

The values obtained before and after treatment in each group were analysed statisticaly as per the standard method described by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The mean values of the blood serum constituents and haemoglobin are presented in Table 1. Though various workers have shown the normal level of haemoglobin to vary between 9-12 gm / 100 ml of blood, but very few reports have been perused concerning its level in different reproductive disorders (Kumar *et al.* 1986). The present findings of haemoglobin concentration in anoestrus animals is in confirmity with that of Dhoble and Gupta (1981). A high significant difference ($P<0.01$) between cycling and anoestrus cows can be concluded that lower level of haemoglobin might be representing some systemic derrangement due to defficiencies of certain trace minerals which in turn has depressed the physiological reproduction. Though the importance of the level of haemoglobin has not been directly implicated in reproductive disorders, yet the decrease in its value is indicative of certain syatemic disorders which can indirectly affect thefunctional activity of the reproductive organs.

The serum copper and zinc concentration of post partum anoestrus and cycling cows (Table 1) is in agreement with the findings of Manicken *et al.* (1977), but is higher than that reported by Sane *et al.* (1958). A significant difference ($P<0.05$) was observed between the values of copper and zinc of cycling and post partum anoestrus cows (Table 1). These variations might be attributed to the inadequate availability of copper and zinc in the feed stuffs. Though a very little is known about the defficiencies of these / trace elements in affecting the complex function of the reproductive system, but it might also be possible that some hormonal activities appear to be correlated with trace elements utilization (Hidiroglou, 1979).

During the present study, out of 36 post partum cows, 24 cows received trace minerals orally for a period of 15 days, of which 18 (75 per cent) animals came to heat within a period of sixty days. Twelve post partum anoestrus cows were kept as control without any treatment, out of which only 4 (33.33 per cent) animals came to heat. The mean value of the blood serum constituents of the experimental and control group are presented in Table 2. In view of the Importance of postpartum anoestrus in cattle various workers have tried diffrent types of mineral supplements but the usefulness of trace minerals like; copper, cobalt, iron and zinc in these animals have been reported only by few workers (Sane *et al.* 1958); Donaldson, 1964 andGupta and Ranjan, 1971).

An increase in the levels of copper, zinc and haemoglobin in the treated group of anoestrus cows with respect to their pre treatment period was found to be highly significant ($P<0.01$) (Table 2), where as in the animals which did not exhibit heat, though there was a little increase in the value of these components before and after treatment the difference was not statistically significant. The findings of significant increase in the level of zinc, copper and haemoglobin in the animals manifested heat is strongly suggestive of deficiencies which has resulted in reducing the functional activity of the genital tract. The non significant change in control animals and animals not responsed to treatment suggests strongly the reason that deficiency of these minerals can be a major factor for anoestrus in cattle.

In the management of cows during post partum period, improper feeding delays the resumption of post partum heat in cows. Further the parturition and lactational stress have a significant effect on the activity of these trace minerals which are responsible in reducing the functional activities of the ovary.

Table 1: Haemoglobin, copper and zinc content of blood / serum of post-partum anoestrus and cyclingcows with the test of significance (Fisher's test) Mean±S.E.)

Blood / serum constituents	Post-partum anoestrus cows (36)	Cycling animals (12)	't' Value
Haemoglobin g%	9.57±0.25	11.74±0.60	3.79**
Copper mcg / 100 ml	101.31±2.74	114.20±6.78	2.05*
Zinc mcg / 100 ml	253.87±13.43	314.00±17.39	2.95*

Figures in parentheses indicate the number of animals

* = (P<0.05)

** = (P<0.01)

REFERENCES

Cellier, H.B. (1955). Haemoglobin (as oxy Hb) estimation in blood. Amer. J. clin. Path., *25:* 221-222.

Dawson, T.B., Ellis, D.J. and Newton, J.H. (1968). Clin. Chem., *21:* 33. In **Clinical Laboratory Method,** by John D. Bauer, 9th 3d., pp. 157..

Dhoble, R.L. and Gupta, S.K. (1981). Total plasma protein and haemoglobin status during oestrus cycle and anoestrus in post-partum buffaloes. Indian vet. J.,*58:* 544-547.

Donaldson, L.E. (1964). A fertility response in cattle following copper therapy. J. Anim. Sci.,*23:* 1233-1237.

Gupta, B.S. and Ranjhan, S.K. (1971). Effect of copper and cobalt supplementation on the reproduction of Murrah heifers. Indian J. Anim. Hlth., *X:* 115-116.

Hidiroglou, M. (1979). Trace element deficiencies and fertility in ruminants. A Review. J. Dairy Sci.,*62:* 1195-1198.

Kumar, S., Shrma, M.C. and Dwivedi, S.K. (1986). Blood Cellular 'Change' in non-descript bovine heifers during anoestrum and repeat breeding conditions. Indian J. Dairy Sci.,*49:* 459-461.

Manickam, R., Gopalakrishnan, C.A., Ramanathan, G., Mookkappan, M. and Nagarajan R. (1977). Studies on the relationships between trace elements and fertility in cows. Indian J. Anim. Res., *11:* 23-26.

Sane, C.R., Deshpande, S.P., Deshpande, B.R. and Kaikini, A.S. (1958). Herd infertility in Gir cattle due to copper deficiencies. Indian vet. J.,*35:* 154-156.

Smith, J.C., Butrimovitz, G.P. and Purdy, W.C. (1979). Clin. Chem., *25:* 1487. In Clinical Laboratory Method, by John D. Bauer, 9th ed., pp. 519..

Snedecor, G.W. and Cochran, W.G. (1967). **Statistical Methods,** 6th ed., Oxford and IBH Publishing Co., New Delhi.

Table 2: Comparision of Copper, Zinc and Haemoglobin concentration of Post-partum anoestrus cows before and after treatment.

Groups	Clinical Observation	Treatment Treatment	Serum Copper concentration mcg / 100 ml	't' value	Serum Zinc concentration mcg / 100 ml	't' value	Haemoglobin gm %	't' value
Treatment Group (24)	Heat manifested (18)	Before	100.72±3.39	3.61**	251.11±10.66	3.43*	9.40±0.45	2.45*
		After	116.72±3.36		300.61±8.59		11.03±0.49	
	Heat not manifested (6)	Before	101.20±13.18	0.12^{NS}	256.00±43.08	0.37^{NS}	9.56.53	0.23^{NS}
		After	108.40±13.67		264.00±45.34		9.72±0.43	
Control Group (12)	Heat manifested (4)	Before	103.42±11.20	0.99^{NS}	257.14±22.08	0.62^{NS}	9.74±0.81	1.36^{NS}
		After	116.28±17.42		302.88±40.33		11.31±0.81	
	Heat not manifested (8)	Before	100.66±9.62	0.46^{NS}	260.00±16.14	0.36^{NS}	9.63±0.38	0.18^{NS}
		After	105.66±0.52		263.33±16.11		9.71±0.27	

Mean±S.E.

Figures in parentheses indicate the number of animals under study.

* = P<0.05

** = P<0.01

IJAR. 16(1), 1995; 32-33

Clinical efficacy of JANOVA in Post-Partum anoestrous Cows

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ABSTRACT

Feeding of JANOVA - a herbal, non-hormonal preparation to post-partum anoestrous cows in an organised farm, helped in oestrus induction in 75% of the 20 treated cows as against 28.6% of contemporary control animals.

Among the treated cows, the success rate was 6/10 in animals having smooth ovaries and 9/10 in cows showing some development of graafian follicles on ovaries.

——x——x——x——

Anoestrus has been reported as the single most frequent reproductive disorder which accounts for 66.6% of infertility problems incows (Roine, 1973). The basic causes of anoestrus in dairy cows are not always apparent and many factors viz. nutrition, gross uterine pathology, hormonal disturbances, chronic debilitating disease and managemental factors are implicated in its causation (Chauhan and Kerry, 1984). It is reported that a majority of reproductive disorders of breedable cows including anoestrus and anovulatory oestrus are of ovarian origin (Singal *et al.* 1988).

Many hormonal and non-hormonal preparations have been used by different workers in the last two decades, to bring anoestrous animals into regular reproductive functioning, with variable response. However, hormonal therapy for anoestrous animals is not considered conducive to the breeding programme for various reasons (Luktuke *et al.* 1977). The present study was undertaken to evaluate the efficacy of a newly developed, polyherbal formulation - JANOVA.

MATERIAL & METHODS

Animals: The trial was carried out on the breedable cows of Shri Gaushala, Bhiwani. Thirty four Hariana and cross-bred (Hariana X Jersey or Hariana X Holstein Friesian) cows calved between August and December 1993, having apparently healthy reproductive genitalia and no clinical infection but without post partum oestrus for 198 to 210 days, were rectally examined and divided into three groups on the basis of their reproductive status as follows:

Group I : Cows having smooth ovaries (10)

Group II : Cows with developing graafian follicles (DGF) on their ovaries but had not shown any mucus discharge or symptoms of oestrus (10).

Group III : Cows kept as control (14). These animals were not tested for their reproductive status and simply kept for observation and monitoring for their oestral behaviour.

All the animals were kept in identical managemental conditions and fed balanced ration having mineral mixture, as per Gaushala practice. Each cow was rectally examined twice with an interval of 8-10 days.

JANOVA AND MEDICATION SCHEDULE

Janova capsule is a combination of potent herbs scientifically formulated for regulating ovarian function (Each capsule contains:

Present Address:
Consultant Veterinarian Shri Gaushala, Bhiwani: (Haryana). 1873/4, U.E. Gurgaon - 122 001.

* Dabur Ayurvet Limited, 22, Site IV, Sahibabad, Ghaziabad (U.P.) - 201 010.

Indravaruni 500 mg., pippaly 50 mg., Marich 50 mg., Sunthi 50 mg. and excipients q.s.).

Each cow was fed 3 capsules per day of Janova with 100 gm. gur at the second examination for two days. The efficacy of the drug was judged from the visual monitoring of observable signs of oestrus, bellowing and jumping over other cows, confirmed by the presence of fern pattern in the mucus of the oestrous animals. The cows not showing oestrus within a month after janova administration were subjected to rectal examination for reproductive status. Janova treatment was repeated for two consecutive days and oestrus appearance, if any, was monitored for the succeding two weeks.

RESULTS AND DISCUSSION

Out of the 10 anoestrous cows having smooth ovaries (Gr. 1), 6 came in heat after feeding of Janova within 30 days of medication.

In Gr. II, out of 10 animals having some fluctuations indicating development of graafian follicles on their ovaries, 9 showed oestrus following janova administration, 6 of them within 30 days. It is likely that the 3 animals which showed oestrus within 31 to 40 days of single treatment schedule, might have come in heat within 20 days, but due to their weak manifestation of oestrus, proper observations might not have been taken by the attendants or the teaser bulls. But as the hypothalamo-hypophyseal ovarian axis stimulated by the feeding of the drug continued functioning more rationally, the animals showed standing oestrus withn 31 to 40 days.

In Gr. III, out of 14 animals only four animals came in heat during the period of study.

All the animals showing oestrus in different groups had copious mucus discharge and conspicuous fern pattern indicating standing oestrus. The results indicated that animals of Gr. I & II had the inherent capacity for normal breeding, but due to hormonal asynchrony in the early post-partum period, they did not show heat. Feeding of Janova seemed to have stimulated the hypothalamo-hypophyseal-ovarian axis thereby synchronising oestrus in 15 (75%) out of 20 such animals.

The animals of Gr. II had shown more response to Janova as out of 10 animals, 7 came in heat with single schedule of janova and another two with two schedules of Janova indicating that slight fluctuations in the ovaries gave good basis for the drug to act for bringing the asynchronous hormonal cycle to a synchronous state by inducing more secretions of hormones either from the hypothalamus or from the anterior pituitary.

The 90% success in Gr. II and 60% success in Gr. I animals showed that Janova successfully induced hypothalamo-hypophyseal-gonadal axis to work, bringing these animals in normal reproductive cyclicity. This was significantly higher than the 28.6% oestrus in the control (Gr. IV). As regards the dosage schedule, it appears that a single schedule (2 doses) may be sufficient where there is some follicular development in the ovaries (70% success). A repeated schedule (4 doses) may be required in animals having smooth overies.

Acknowledgement: The author wishes to express his gratitude to the President, Administrator and the Board of Trustees of Shri Gaushala Trust, Bhiwani for grant of permission to test the drug in the Gaushala animals. The professional assistance rendered by Dr. D.D. Sharma, Administrator and Sh. Mohan Lal, Livestock Development Assistant of Gaushala farm during the course of this study is sincerely appreciated.

REFERENCES

Chauhan, F.S. and Kerry, B.M. (1984). Recent advances in hormonal therapy of bovine reproductive disorders - A review. *Vet. Bull.54:*991-1009.

Luktuke, S.N. (1977). Gynaecological studies in rural cattle & buffaloes and measures for augmenting their breeding efficiency. Ind. Dairyman*29:*281.

Roine, K. (1973). The most frequent reproductive disorders in cows and their seasonal variation. Nord. Vet. Med.*25:*242-247.

Singal, S.P., Lohan, I.S. and Arora, K.L. (1988). Studies on the incidence and reproductive management of intertility in breedable dairy animals. Paper presented in 11th International Cong. on Animal Reprod. & A.I., Dublin (Ireland) 26-30th June, 1988.

IJAR. 16(1), 1995; 34-35

Treatment of Heifer Infertility on Organised Farms

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ABSTRACT

Sixty three crossbred heifers between the age group of 19 to 31 months with the history of anoestrus condition were synchronised for oestrus. Out of 63 heifers 20 were cyclic as determined by rectal palpation and were allotted to PGF2 alpha treatment and rest non-cyclic 43 heifers were allotted to PRID (21) and synchromate B (22) for induction cum synchronization treatments. Besides these 27 heifers were left untreated.

It was observed that 65% of the treated heifers conceived up to 97 days and 76% up to 160 days post treatment corresponding figures for untreated group were 48.13 and 81.45 percent. It was inferred that judicious use of exogenous harmonal treatments can be effectively used for requlariSing of oestrus cycies and thereby handling of infertility problems in organised farms.

——x——x——x——x——

The use of artificial insemination in cattle is getting increasingly popular with farmers on organised farms. Heat detection is more difficult in organised farms because of lack of proper identification of animals, poor expression of heat and dependance on the herdsmen. In order to breed the heifers in time and make replacements available for cows it is essential to adopt certain reproduction management practices so that the problems are minimised. One such experiment was undertaken in organised farm where the problem of infertility in heifers was experienced.

MATERIALS AND METHODS

Ninty crossbred heifers in age group of 19 to 31 months and having more than 50% exotic inheritence were presented for such trial at Military Farms, Manjri and Pimpri under Southern Command. These heifers were examined per rectum for reproductive health, cyclicity etc. All heifers had good body condition and had more than 275 kg body weight on an average. Those heifers which were found to be cyclical were treated with Prostaglandin F2 alpha and those noncyclical were allotted to PRID or synchromate B plus treatment. The protocols followed for different treatments were as under:

1. Prostaglandin F2 alpha - 2 injections of 25 mg were given, 11 days apart and AI was done after 72 and 96 hours of last injection.
2. PRID was inserted in vagina with special applicator on day 'O' and kept till 10th days. PMSG 400 IU and PGF2 alpha 25 mg were injected I/M on day 8th and device was withdrawn on day 10th followed by AI on 48 & 72 hours of withdrawal of device.
3. Synchromate B plus. The norgestomet ear implant was placed under the skin behind ear on day 'O' with 2 ml of synchromate B, intramuscular injection containing 3 mg of norgestomet and 6 mg of estradiol benzoate. PMSG 400 IU and PG 25 mg was given intramuscularly on day 8th. Implant was withdrawn on day 10th followed by AI on 48 & 72 hours of withdrawal.

Twenty heifers were found to be in cycle hence were allotted to PGF2 alpha treatment. 43 animals irrespective of cyclicity were randomly allotted to PRID (21) and synchromate B (22) treatment and rest 27 heifers were left untreated.

RESULTS AND DISCUSSION

It is noticed that under PGF2 alpha treatment 18 heifers remained pregnant within 97 days (90%) following treatment while one heifer (5%) conceived between 98 to 118 days. 14 heifers under PRID and 9 heifers under synchromate

B plus conceived within 97 days showing 66.6% and 41.0% conception rate respectively. 5 heifers under PRID (23%) and 6 heifers under synchormate B (25.4%) took more than 97 days after treatment for conceptions. The untreated group of heifers had 13 conceptions (48%) below 97 days from day of treatment which was appreciably less than the treated group 11 heifers concieved after 97 days (41%) from untreated group which was higher as compared to the treated group.

One heifer under PG, 2 under PRID and 7 under synchromate B did not concieve and were culled from the herd. Similarly 3 heifers from untreated group were culled from the herd on account of the similar reasons.

Hewett (1968) found significant relationship of heat detection efficiency with herd size, the larger the herd the lower the estrus rate. The herds of crossbred animals at military farms are bigger and perhaps heat detection inheifers would be difficult. Bhosrekar *et al.* (1994) obtained higher conception rate in heifers synchronised with PGF2 alpha as compared to Norgestomet implants which is in total agreement with the present finding. French workers Petit *et al.* (1977) and Petit *et al.* (1984) found similar results with PGF2 alpha treatment in European cattle heifers.

Acknowledgement: Authors wish to thank the Prsident of BAIF Development Research Foundation for the keen interest in the work, so also to the authorities of Military Farm for their co-operation.

REFERENCES

Bhosrekar M.R., Y.P. Phadnis and B.R. Mangurkar. Oestrus behaviour and conception rates inoestrus synchronised cattle and buffalo heifers (Under publication) Indian Journal of Animal Science.

Hewett D.C. (1968) cited by Bhosrekar, M.R. In Investigation into the incidence and cause of repeat breeding in dairy cattle at NDRI, Karnal Haryana. Indian Vet. Journal *50:(5)* 1973. 418-429.

Petit, M., Baye, M. and Palin C. (1979). Maitrise des cycles sexules. Elevage at Insemination *170:*7-29.

Petit M. Chupin, D and Pelot, J. 1977. Synchronization des chaueurs chez les genises de race Laiteere In : Compet Randu des jounes 11 EB ONCEIA ITEB

IJAR. 16(1), 1995; 36-38

Insolation, Indentification and Sensitivity pattern of Microbial agents from cases of Clinical Endometritis

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ABSTRACT

Microbial agents were isolated from 21 cases of clinical endometritis and recorded the antibiotic sensitivity pattern of the bacterial isolates. The isolates were *staphylococcus aureus* (14.28 per cent), Coagulase negative *staphylococci* (4.76 per cent). Antibiotic sensitivity test revealed that maximum per cent of animals were sensitive to gentamicin followed by Chloramphenicol, Oxytetracycline, Sulphadiazine and Nitrofurantoin, whereas maximum resistance was to penicillin and streptomycin.

——X——X——X——X——

An investigation was carried out to isolate the common microbial agents associated with cases of clinical endometritis and to study their antibiotic sensitivity pattern.

MATERIALS AND METHODS

Materials for the study consisted of twenty one crossbred cows belonging to the livestock farm Mannuthy attached to the Kerala Agricultural University. All cows which did not conceive beyond 45 days post partum were subjected to detailed clinico-gynaecological examination. Those found to have clinical endometritis as evidenced by aberrations of oestrus, abnormal discharge and a large doughy uterus were selected.

Aseptic collection of uterine discharge was carried out by using an instrument fabricated by Vahida (1992). The sample collected was transferred to a collection vial and used for isolation of the organisms and for antibiotic sensitivity tests. A portion of the sample was streaked on Mueller-Hinton agar by streak plate method in order to get well isolated colonies of bacteria present in the sample and incubated at 37°C for 18 to 24 hours. One half of a well isolated single colony was subjected to Gram's staining and other half was subcultured on Mueller-Hinton agar for the purification of the organism. Antibiotic sensitivity tests were performed using few well isolated colonies of actively growing organisms obtained from each sample (Barry, 1976). Isolation and identifications of organisms were attempted with 21 samples. Identification of the most predominantly occuring isolates from each sample was elucidated on the basis of morphology and staining reactions, oxidation-fermentation of glucose, growth on Mac-Conkey media, Catalase test, oxidase test, motility, haemolysis on blood agar, Coagulase test, pigment production and further by a battery of biochemical tests (Cowan, 1974).

RESULTS AND DISCUSSION

Uterine discharge from 21 cases of clinical endometritis were subjected to isolation and identification of bacterial organisms, antibiotic sensitivity tests and the results are given in table 1 and 2 respectively. The present study isolated coagulase negative staphylococcus species (9.52 per cent) *staphylococcus aureus* (14.28 per cent), Bacillus Spp. (23.84 per cent) Corynebacterium Spp. (9.52 per cent) Pseudomonas Spp (14.28 per cent) Citrobacter Spp. (23.84 per cent) and *Candida guilliermondii* (4.67 per cent). Hinze (1959) opined that non specific opportunist pathogens are the most common causes of endometritis and that these organisms might have reached the uterus from vagina at oestrus or at parturition Khan *et al.* (1990), Biolatti (1991) and Vahida (1992) also isolated common microbial organisms from

* Part of M.V.Sc Thesis submitted to the Kerala Agricultural University, Mannuthy, by first Author.

the uterine exudate as reported in the present study. However, perusal of literature did not reveal isolation of *Candida guilliermondii* as reported in the present study. This organism was isolated from uterine exudate of one cow in which discharge did not become clear even after treatment with PGF_2 alpha. Probably further studies on this organisms and its treatment are warranted.

A critical study on the sensitivity test of the uterine exudate revealed that maximum per cent of animals were sensitive to gentamicin followed by chloramphenicol, Oxytetracycline, Sulphadiazine and nitrofurantoin. On the other hand maximum resistance was to penicillin and streptomycin. Varadarajan and Nair (1989), Iyer (1992) and Vahida (1992) also reported that gentamicin was the most effective drug for clinical endometritis. The findings that majority of animals were resistant to penicillin and streptomycin was reported earlier also (Iyer, 1992). This might be on account of the indiscriminate use of penicillin and streptomycin in treating infections making them resistant to these antibiotics.

Tabel 1. Organisms isolated

Sl.No.	Organisms identified	Number	Per cent
1.	*Staphylococcus aureus*	3	14.28
2.	Coagulase negative Staphylococci	2	9.52
3.	Bacillus Spp.	5	23.80
4.	Corynebacterium Spp.	2	9.52
5.	Psudomonas Spp.	3	14.28
6.	Citrobacter Spp.	5	23.84
7.	*Candida guilliermondii*	1	4.76
		21	**100.00**

Table 2. Response to sensitivity tests

Sl. No.	Antibiotics/ Antibacterials used	Sensitive		Resistant		Total
		No.	percent	No.	percent	
1.	Pencillin	4	20	16	80	20
2.	Streptomycin	5	25	15	75	20
3.	Oxytetracycline	11	55	9	45	20
4.	Chloramphenicol	14	70	6	30	20
5.	Sulphadiazine	11	55	9	45	20
6.	Nitrofurantoin	10	50	10	50	20
7.	Gendtamicin	16	80	4	20	20

REFERENCES

Barry, A.L. (1976). *The antimicrobial susceptibility test: Principles and Practices,* Llea and Febiger, Philadelphia, pp. 72-80.

Biolatti, B. Bollo, E., Quarants, G., Vincient, L., Luini, M. Perini, S. and Fabbi, M. (1991). Biopsy, virological and bacteriological studies on the endometrium of cows with low fertility. *Atti della Societa Haliana di Buiatria* **23:**139-146. Cited in *Vet. Bull.* (1992) **62**(5):2715.

Cowan, S.T. (1974). *Manual for th identification of medical bacteria* University Printing House, Cambridge, Great Britain, 2nd Edn. pp. 11-186.

Hinz, P.M. (1959). Diagnosis and treatment of non specific infertility in the dairy cow. *J. Am. Vet. Med. Ass.* **134**(2):302-307.

Iyer, C.P.N., Nair, K.P. Susdarsanan, V., Madhavan, E., Mathai, E., Nair, M.S., Vijayakumar, V. and Joseph, M. (1992). Reproductive disorders of cross-breed cows of Kerals. *Indian. J. Anim. Reprod.* **13**(1) 65-68.

Khan, M.A., Hussain, I. Ashfaque, M. and Ahamad, K.M. (1990. Studies on the bacteriology of Metritis with special referenceto Brucella in buffaloes and cows. *Pakistan. Vet. J.* **10**(4):157-168. Cited in *Vet. Bull.*(1992) **62**(9) : Abst. 5785.

Vahida, A.M. (1992). Treatment of endometritis for improving fertility in dairy cows. *M.V.Sc. Thesis, Kerala Agricultural University.* pp. 1-63.

Varadarajan, M. and Nair, K.P. (1989). Efficacy of intrauterine infusion of gentamicin alone or in combination with stilboesterol in the treatment of endometritis in cow *J. Vet. Anim. Sci.* **21**(1) : 83-86.

IJAR. 16(1), 1995; 39-41

Factors Affecting Service Period in Holstein-Friesian and Jersey Purebreds

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ABSTRACT

The study included 657 service period records on purebred Holstein Friesian and Jersey cows maintained in the same herd and management regime. The least-square mean service period was observed to be 177.91±37.64 and 125.23±17.88 days in Holstein Friesian and Jersey cows respectively. The effect of parity on service period was non-significant whereas the effect of season of calving was statistically significant in both the breeds. The haemoglobin level, body weight at 3 months post-partum, 100 day milk yield and body weight gain after calving influenced the service period in Holstein Friesian cows only. Effect of these factors in Jersey was statistically non-significant. It was inferred that the Holstein Friesian cows might be more sensitive than Jersey cows to various physiological and environmental stresses which affect the reproductive efficiency.

——x——x——x——

Service period is the most important reproduction trait of economic importance in dairy cattle, and yet very little is known about its manifestation and control in exotic breeds and their cross-breds maintained in Indian conditions. Holstein Friesian and Jersey are the two commonest exotic dairy breeds used for crossing indigenous females and therefore a comparative study of service period in these two breeds in India makes a special significance.

MATERIALS AND METHODS

Data included 321 and 336 service period records of Hostein Friesian and Jersey cows respectively, along with corresponding observations on parity, calving dates, the body weights at calving, at 3 months and at 6 months post-calving; Haemoglobin (gm%) estimation at or near to date of body weight record; and 100-day milk yield of ongoing lactation during the period from year 1982 to 1989.

Least-square analysis was carried out with service period as the dependant variable; lactation sequence, season of calving as grouping (independant) variables; and the Haemoglobin level and body weight at 3 months post-partum, 100-day milk yield, gain in body weight from calving to 3 months and from 3 months to 6 months as the covariates.

RESULTS AND DISCUSSIONS

Calving interval in cattle can be divided into two components, namely the service period and the gestation length. The service period was chosen as the target component for study of variability. The variability in service period might again be attributed to variation inseveral other genetic and non-genetic factors. The means and measures of dispersion of these variables is in Table - 1.

The mean service period in HF and Jerseycows was observed to be 170.0±88.2 and 127.0±69.9 days respectively. The difference was highly significant which indicated that the Jersey exhibited significantly higher reproductive efficiency as compared to JF maintained in the same herd management regime. The observation of this study were in agreement with Mc Dowell *et al.* (1974) who reported that

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Jerseys were apparently better than HF in respect of reproductive efficiency.

The dairy breeds loose the body condition commensurate with the stress of lactation. In the present data, the loss of body weight during 3 months post partum calving averaged around 20 kg and the variability in gain (or loss) was very high as evinsed by the magnitude of standard deviation. Similarly, the gain in body weight during the subsequent 3 months was only marginal of about 4 kg and again with high standard error. These observations tendend to show that the loss of body weight and recoupment was heavily dependant on individuality of cows.

Least-square analysis of variance of service period is presented in Table — 2. The least-square mean service period in HF and Jersey was observed to be 177.91±37.64 and 125.23±17.88 days.

It was observed that the effect of parity was non-significant whereas the season of calving significantly influenced the following service period both in HF and Jersey. The least-square means of service period in JF were 186.77±10.78, 156.81±11.53 and 190.15±11.43 days for rainy season (June to September), Winter (October to January) and Summer (February to May) calvings respectively. The corresponding means in Jerseys were 137.05±7.64, 112.06±7.75 and 126.58±7.06 days in the same order. It meant that the calvings between October to January were most favourable to minimise the service period. This favourable impact might be attributed to several factors such as improved forage availability, comfortable climatic conditions, better expression and detection of heats in the following season.

Mangurkar *et al.* (1985) observed that effect of season and breed was significant whereas effect of period and parity was non-significant in HF and Jersey purebreds.

Haemoglobin level, body weight at 3 months post-partum, 100 days milk yield and body weight gain influenced the service period in Holstein Friesian cows only. Effect of these factors was statistacally non significant in Jersey cows. These observations tended to show that Holstein Friesian cows might be more sensitive to physiological and environmental stresses which affect their reproductive performance.

Chaudhary *et al.* (1989) observed that service period of both Holstein x Sahiwal and Jersey x Sahiwal crosses was affected by season, year of calving and parity whereas Jadhav and Bhatnagar (1987) reported significant influence of lactational milk yield on the service period. Tahir and Marrof (1988) revealed that the effect of sire, age at first calving, parity and 305 day milk yield on service period was non-significant whereas the effect of calving year was significant. Methekar *et al.* (1993) reported that location and period had significant influence on service period while season of calving had a non-significant effect on service period in Jerseys. The variation in the observed effects from the literature indicates that the effects of environmental factors on reproduction parameter are usually specific to locations, breeds, time and management regime. Consequently, generalisations of the results is warranted. Neverthless, the above studies on comparative performance of purebred HF and Jersey were in contemporary situation and hence focussed special attention on the differential response.

Table 1: Means and Measures of Dispersion

		Breed			
		HF (N = 321)		JERSEY (N = 336)	
		Mean	S.D.	Mean	S.D.
1.	Service Period (days)	170.0	88.2	127.0	69.9
2.	Haemoglibin at 3 months (gm%) post calving	7.43	0.88	8.00	1.19
3.	Body weight at 3 months post calving (kg)	464.9	53.9	336.0	36.0
4.	100 days Milk Yield (kg)	1670.2	369.2	1264.4	298.8
5.	Gain in body-wt. from calving to 3 months (kg)	(—)18.2	35.6	(—)19.5	30.0
6.	Gain in body-wt. from 3 to 6 months (kg)	4.2	25.3	4.3	19.0

Table 2: Least-square analysis of variance of service period in Hostein Friesian and Jersey cows.

Source of variation	Holstein Friesian		Jersey	
	d.f.	Mean square	d.f.	Mean square
Parity	7	8,849	8	6,187
Seasons	2	34,220 **	2	15,115*
Hemoglobin at 3 months	1	28,241 **	1	2,112
Body wt. at e months post-partum	1	29,976 **	1	13,652
100 days milk	1	71,830 **	1	11,965
Body weight gain from calving to 3 months	1	30,208 **	1	2,409
Body weight gain from 3 to 6 months postcalving	1	164,839 **	1	9,798
Residual	306	6.611	320	4,683

(Note: * (P<05, ** (P<0.01)

REFERENCES

Chaudhary, M.A, Amer, M.A. and Bukhari, S.M. (1989). Nongenetic factors affecting service period and services per conception in half bred cows. Pakistan Veterinary Journal *9(1) :* 20-27.

Jadhav, K.L and Bhatnagar, D.S. (1987). Effect of production level on production traits in cross-bred cows. Indian Journal of Animal Research *21 (2):* 107-108.

Mangurkar, B.R., Gokhale, S.B., Shinde, D.N., Pande, A.B. and Phadnis Y.P (1985). Reproduction performance of Holstein Friesian and Jersey purebred cows in a herd in India. Indian Journal of Animal Sciences. *55 (10):* 893-897.

Mc Dowell, R.E., Velasoo, J.A., Van Vleck, L.D., Johonson, J.C., Brandt, G.W., Hollan, B.F. and Mc Daniel, B.T. (1974). Reproductive efficiency of purebred and crossbred dairy cattle. Journal of Dairy Science *57 (2):* 220-234.

Methekar, K.U., Deshpande, A.D. and Despande, K.S. (1993). Factors affecting service period and calving interval in Jersey cows. Indian Journal of Dairy Science, *46 (10):* 496-497.

Tahir, K.N. and Marrof, N.N. (1988). Studies on the performance of Friesian cattle in Iraq III. Service period and calving interval. ZANCO *6 (4):* 41-47.

IJAR. 16(1), 1995; 42-43

Variations In Serum Macro Minerals During Different Stages of Reproduction in Goats

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ABSTRACT

Changes in serum calcium, inorganic phosphorus, magnesium, sodium and potassium during different reproductive stages ie. on the day of oestrus, early pregnancy, mid pregnancy, late pregnancy and non-pregnancy, were studied n indigenous goats of Assam. The serum inorganic phosphorus and magnesium levels showed peak values on the day of oestrus. These values were significantly ($P<0.01$) different than the other stages of reproduction. However, serum calcium, sodium and potassium did not vary significantly during different stages of reproduction.

——x——x——x——

Minerals are not only participating as cofactor, and activators of various enzymes in the carbohydrate and energy metabolism, but also associated with normal reproductive behaviour in domestic animals. Low levels of circulatory minerals results in impaired reproductive functions leading to cessation of cyclic activity (Martson, *et al.* (1972). Moreover, these elements fluctuates during different stages of reproduction. But there is a paucity of information on the changes of various levels of macro elements in goat during there different stages of reproduction. Therefore, the present experiment was conducted.

MATERIALS AND METHODS

Experiment was conducted on groups of fifteen female indigenous goats of Assam (Capra 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 nonpregnancy 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 separated. Serum samples were subjected to determination of macro minerals. Calcium, inorganic phosphorus, magnesium, sodium and potassium 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 calcium, inorganic phosphorus, magnesium, sodium and potassium as recorded in serum of goats indifferent stages of reproduction are presented in table.

In this study, although the serum concentrations of Ca did not differ significantly during different stages of reproduction, but the level showed an apparently increasing trend from the day of oestrus towards late pregnancy, which might be due to higher demand of Ca for the growing foetus at late pregnancy. The lower level of Ca, obsrved on the day of oestrus, may be attributed to oestrogen, which may change the appetite, and hence Ca intake, resulting in diminished Ca absorption (Bar *et al.* 1971; Sivaiah *et al.* 1984 and Nigam *et al.* 1990).

Significantly higher levels ($P<0.01$) of P on the day of oestrus and in late pregnancy (Table) might be due to, firstly, elevated oestrogen, which

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raises the level of phosphatase subsequently. Secondly, the rate of phosphorus exchange in tissuses increases during periods of reproductive activity. During the second half of pregnancy, the absorption of phosphorus by animals and its deposition in the skeleton increases. Endogenous excretion of phosphorus decreases and phosphoraemia increases. In this way, reserves of phosphorus are built up in the mother's body and are then utilized for the growh of foetus and in subsequent lactation (Georgievskii, 1982).

The concentration of Mg was also significantly higher (P<0:01) on the day of oestrus than in subsequent reproductive stages. The higher level of Mg on the day of oestrus could be explained in the similar line as described in respect of P. Similar result was also reported by Sahukar *et al.* (1984).

The levels of Na and K, as observed in the present study,did not vary significantly. These findings are inclose agreement with the findings of Bar *et al.* (1971) and Baruah *et al.* (1988).

Table: Levels of serum calcum, Inorganic phosphorus, Magnesium, Sodium and Potassium during different reproductive stages in goat.

Reproductive stages	Minerals				
	Calcium (mg / 100ml)	Inorganic phosphorus (mg / 100ml)	'Magnesium (mg 100ml) 100ml)	Sodium (meq / L)	Potassium (Meq / L)
On the day of oestrus	9.40±0.30	8.21^{a}±0.31	3.72^{a}0.09	144.58±6.57	4.93±0.12
Early pregnancy	9.66±0.38	6.72^{b}±0.37	2.72^{b}0.15	149.50±4.80	5.00±0.17
Mid pregnancy	9.75±0.50	6.77^{b}±0.41	2.68^{b}±0.12	148.67±3.50	5.10±0.25
Late proegnancy	10.28±0.43	7.32^{c}0.42	2.63^{b}±0.17	147.42±5.04	5.23±0.21
Non-pregnancy	9.56±0.21	4.55^{d}±0.10	2.62^{b}0.17	150.74±2.18	5.30±0.19

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

REFERENCES

Bar, H.J.; Seidel, H. and Muller, J. 1971. Minerals concentration in the serum of clinically healthy cattle. Vet. Med. *24;* 889-892.

Baruah, P.M.; Dutta, J.c. and Rajkonwar, C.K. 1988. Serum sodium and potassium levels during oestrus cycle in cows. Indian Vet. J. *65 (2);* 1155-1156.

Georgievskii, V.I.; Annenkov, B.N. and Samokhin. 1982. Minerals nutrition of animals. Kolos publishing House, Moscow. English translation — Butterworth and Co. (publishes) Ltd. Witham, Essex.

Martson, H.R.; Shirley, H.A. and Smith, R.M. 1972. "Production within the rumen and removal from the blood stream of VFA in sheep given a diet deficient incobalt". Brit.J. Nutri *27;* 147

Nigam, R.; Patel, A.V.; Sureskumar, P.K.; Patel, D.M. and Mehta, V.M. 1990. A study on the levels of serum calcium and phosphorus during oestrous cycle in Surti and Marwari goats. Indian J. Amim. Res. *24 (1);* 52-54.

Sahukar, C.S.; Pandit, R.K.; Chauhan, R.A.S. and Porwat, M.L. 1984. Electrolytes during various reproductive phases in crossbred cows. Indian J. Anim. Sci. *54 (10);* 993-995.

Sivaiah, K.; Rao, K.B. and Murthy, A.S. 1984. Serum calcium and inorganic phosphorus levels in different stages of reproduction in crossbred cattle. Abst. of the symposium on redent advances inmineral nutrition, Haryana Agricultural University, Hissar.

Snedecor, G.W. and Cochran, W.G. 1967. Statistical methods, 6th edn. Iowa State University Press, Ames.

IJAR. 16(1), 1995; 44-46

Studies on Biochemical Constituents of Blood in Anoestrus, Repeat Breeding and Cyclic Indigenous Sows (Sus Scrofa Domestica)

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ABSTRACT

Eighteen indigenous sows were distributed into three groups of six each on the basis of reproductive status, viz. anoestrus, repeat breeding and cyclic. Blood samples were collected for estimation of glucose, cholesterol, calcium, inorganic phosphorus and total protein. The average blood glucose, cholesterol, calcium and phosphorus were higher in cyclic sows. Blood glucose and cholesterol values varied significantly. Serum protein did not show any trend with regard to the three groups. In general, it indicated that blood glucose, cholesterol, calcium and inorganic phosphorus values affected the reproductive process.

——x——x——x——

The prosperity of pig industry depends upon the breeding efficiency of swine. However, there are certain handicaps in pig breeding. The major infertility problems encountered in this species are anoestrus, repeat breeding and unobserved oestrus. Data representing the biochemical profile as a comparative measure appear to be totally lacking especially for indigenous sows.

MATERIALS AND METHODS

Eighteen indigenous sows maintained at the All India Co-ordinated Project on Pigs, Livestock Farm, Adhartal, Jabalpur (M.P.) under the conventional feeding and managerial conditions were selected for the study. These indigenous sows were divided into three groups of six each on the basis of their reproductive status as under:

Anoestrus Group

Under this group, such sows were included which failed to exhibit any overt signs of oestrus for the last six months.

Repeat Breeding Group

This inlcuded sows mated to a known fertile boar during five consecutive oestrous cycles but failed to conceive.

Normal Cycling Group

This group included sows exhibiting standing oestrus behaviour with acceptance of the male.

Blood samples were collected aseptically from all the sows in two test tubes with and without anticoagulant (Dipotassium EDTA). Uncoagulated blood samples were immediately used for bloodglucose estimation (Folin and Wu, 1920). Serum harvested from coagulated blood was used for estimation of total cholesterol (MacIntyre and Ralston, 1954), total protein (Greenberg, 1929), calcium (Weli and Wiedeking, 1970) and inorganic phosphorus (Fiske and Subbarow, 1925). Chi square test was applied for comparison of respective means.

RESULTS AND DISCUSSION

Mean and Standard errors for blood glucose, cholesterol, calcium, inorganic phosphorus and total protein have been presented in table 1.

Blood Glucose

Blood glucose differed significantly between groups. A low glucose level in anoestrus and repeat breeder indigenous sows was observed as compared to normal cycling sows. The normal

range of blood glucose for exotic sows has been reported as 85 to 150 mg/100 ml (Kaneko, 1980). In this study lower levels were obtained (Table 1) even in the normal cycling sows. Low blood glucose has also been reported in anoestrus buffaloes (Derashri *et al.* (1984; Chouhan, 1993) and repeat breeder cows (Awasthi, 1987) as compared tonormal cycling animals.

McClure (1965) postulated that the secretion of gonadotrophins may either be reduced or stopped due to hypothalamic failure in utilizing glucose thus affecting the hypophyseal activity. Thus, low level of blood glucose in anoestrus animals may show a subnormal energy status. Pituitary function appears to be particularly influenced by blood glucose level. Follicular Stimulating Hormone (FSH) is a glycoprotein, the function of which is influenced by its carbohydrate moiety (Hafez, 1969). Hence, significantly low blood glucose in anoestrus and repeat breeding sows is logical. Despite this, overall lower profile of blood glucose in the indigenous sows used in this study as compared to the data available for exotic breeds (Kaneko, 1980) may explain the low prolificacy these animals over their exotic counterparts.

Total Cholesterol

Serum cholesterol concentration was significantly lower in anoestrus and repeat breeding sows as compared with normal cycling indigenous sows (Table 1). The normal range of total cholesterol in exotic sows has been reported as 26-54 mg / 100 ml (Kaneko, 1980). In this study, the values were much higher in all the groups. High serum cholesterol has also been reported in the bovine estrous cycle during the follicular phase (Fillios and Mann, 1956). Estrogen effects the carbohydrate metabolism which in turn increases the production of cholesterol in the endocrine gland tissue from acetate. Thus, the values were significantly higher in the normal cycling sows. Significant difference has also been reported in total serum cholesterol level in fertile and non fertile cows (Kumar *et al.* 1986) which corroborates with the similar trend between fertile and non fertile sows as obtained in ths study.

Calcium and Inorganic Phosphorus

Serum calcium and inorganic phosphorus were higher in the normal cycling sows as compared with the repeat breeder and anoestrus sows but the difference between these groups were non significant. The normal serum calcium and inorganic phosphorus levels in the exotic sows has been reported as 9.65±0.99 (range 7.1-11.6 mg / 100 ml and 5.3-9. 6 mg / 100 ml respectively (Kaneko, 1980). The turn over of calcium in the body not only depends on sufficient total dietary supplies, but also on chemical forms in which they occur in the diet and vitamin D status of the diet or the animal. Inorganic phosphorus has been shown to be significantly lower during oesturs in sows as compared to the other days of the cycle (Laszyk *et al.* 1979) which is contrary to the findings in this study. Repeat breeding has also been attributed to inorganic phosphorus deficiency in cows (Dzanasija and Salalov, 1968; Morrow, 1969).

Total Protein

Anoestrus sows had a higher level of serum protein as comapred to repeat breeding and normal cycling sows though the difference was non significant. Roberts (1971) opined that protein deficiency of either quantity or quality under usual conditions are not common except under severe inanition or underfeeding which justifies the non significant results obtained between the various groups of sows in this study for this parameter.

Thus, in the present study, contents of blood glucose, total cholesterol, calcium and phosphorus appeared to significantly affect the reproductive performance of the indigenous sows.

Acknowledgement: Authors are highly indebted to the All India Co-ordinated Research Project on Pigs, Livestock Farm, Adhartal Jabalpur for providing experimental animals for the present study and to Department of Veterinary Biochemistry, College of Veterinary Science and Animal Husbandry, Jabalpur (M.P.) for all the assistance in biochemical estimations.

Table 1: Mean±Standard error of biochemical constituents of blood in anoestrus repeat breeding and normal cycling indigenous sows.

Groups	Blood glucose (mg / 100 ml) (mg / 100 ml)	Total Cholesterol	Calcium (mg / 100 ml) (mg / 100 ml)	Inorganic Phosphorus (gm / 100 ml)	Total Protein
Anoestrus	51.97±5.97*	77.45±8.92*	7.91±2.2	6.47±1.92	9.13±1.03
Repeat Breeder	62.54±4.46*	87.32±6.12*	9.44±3.10	7.97±1.12	8.23±0.37
Cyclic	82.49±6.32	108.91±3.51	11.21±2.59	8.04±1.21	8.55±0.29

* Significant at 90% confidence level (Chi square test).

REFERENCES

Awasthi, M.K. (1987) Studies on incidence, associated causes and remedial measures in repeat breeder cross bred cows. M.V.Sc. & A.H. thesis, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur.

Chouhan, (1993). Certain studies on genitalia, follicular fluid and blood of buffaloes. M.V.Sc. * A.H. thesis, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur.

Derashri, H.J., Kodagali, S.B., Kurmi, F.. and Nema, S.P. (1984). Anote on trial with Gentavet in repeat breeders. Indian J. Anim. Reprod. *1:* 66.

Dzanasija, H.T. and Salalov, G (Jr.) (1968). Minerals for prevention of infertility in cows. Nutr. Abst. Rev. *38:* 6222.

Fillos, L.D. and Mann, G.V. (1956). Circulation Research. 4: 406. Cited by Murthy, T.S. and Rao, N.A.V. (1981). Blood cholesterol profiles in different reproductive phases of graded Murrah buffaloes. Indian Vet. J. *58:* 771-773.

Fiske, C.H. and Subbarow, Y. (1925). The colorimetric determination of phosphorus. J. Biol. Chem., *66:* 375-374.

Folin, O. and Wu, H. (1920). A system of blood analysis. Supplement 1. A simplified and improved method for determination of sugar. J. Biol. Chem., *41:* 367-374.

Greenberg, D.M. (1929). The colorimetric determination of serum protein. J. Biol. Chem., *82:* 545-550.

Hafez, E.S.E. (1969). Cited by Murthy, T.S., Sreemalu, P. and Pillai, K.V. (1981) Studies on blood glucose levels in different physiological states. Livestock Advisor *9(6):* 43-48.

Kaneko, J.J.(1980). Ed. Clinical Biochemistry of Domestic Animals. Acad. Press, N.Y.

Kumar, S., Sharma, M.C. and Dwiwedi, S.K. (1986). A note on the changes in haemoglobin and certain biochemical constituents of blood serum during fertile and non fertile oestrus inrural cows. Sixth National Congress on Animal Reproduction, Assam Agriculatural University, Guwahati. pp. 1.

Laszyk, j., Candarle, j., Dvorak, M., toulova, M., Matouskova, . (1979). Biochemical and haematological changes in sows during oestrus. Veterinarni Medicina *24:* 311-316. (Vet. Bull. 50: 424).

Mac-Intyre, 1. and Ralston, M. (1954). Micro-analysis in medical biochemistry (King, E.J. and Wooten, I.D.P.), J. and A. Churchill Ltd., London.

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

Morrow, D.A. (1969). Phosphorus deficiency and infertility indairy heifers. J. Am. Vet. Med. Assoc. *154:* 761-768.

Roberts, J. (1971). Veterinary Obstetrics and Genital Diseases (Theriogenology). 2nd. Ed. Scientific Book Agency, Calcutta.

Weli, B. and Wiedeking, E. (1970). Determination of trace elements in serum and uring using flameless atomization. Fresenius Zeitschrift fur anlytische Chemie, *252:* 111-117.

IJAR. 16(1), 1995; 47-49

Study of Some of the Reproductive Traits of Kathi Mares in Gujarat State

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ABSTRACT

A study was carried out on 125 foaling records of 24 Kathi mares maintained at Govt. Horse Breeding Farm, Junagadh (Gujarat) to know the age at first service and foaling, gestation, period, interfoaling period and foal heat days. The mean age at first service was 1714±78 days and age at first foaling was 2106±92 days. The mean gestation length was 331±1days with an average interfoaling period of 460±16 days. The average foal heat period was 10 days. The number of services per pregnancy averaged 1.62. The fertility rate at foal heat was 53.03 percent. The maximum breeding took place during the months of May, June and July with comparatively higher breeding frequency from March to September. Gestation length was apparently more in mares covered during high breeding season (331.38±0.87 days) than those covered in low breding season (327.38±2.27 days). Parity had no effect on any of the traits studied. The birth rate of fillies was nonsignificantly higher (51.2%) than the colts (48.8%). Mares carrying male foals had longer gestation period (331.56±1.17 days) than those carrying female foals (329.91±1.16 days), the difference being nonsignificant. The placental weight was significantly higher (3.96±0.13 kg) in mares conceived during high breeding season than in low breding season (337±0.10 kg). Similarly, the mean birth weight was higher (39.88±0.74 kg) in high breeding season than in low breeding season (39.36±1.06 kg), differing nonsignificantly.

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The mare is unique among domestic animals undergoing a fertile oestrus shorty after parturition. Because of man's exploitation of horse for pleasure and racing, the pressure within the breeding industry has mounted to get as many as mares pregnant at the earliest possible within the breeding season. This has lead to the practice of breeding the mare at the first post-partum oestrus (foal heat) with an average Throughbred gestation of 340 days (Ginther, 1979; Lay, 1980). Very limited information is available on Kathi mares pertaining to their reproductive traits including fertility. Hence, an affort has been made to analyse the pertinent reproductive records of Kathi mares stationed at Govt. Horse breeding Farm, Junagadh, Gujarat State.

MATERIALS AND METHODS

A total of 125 foaling records of 24 Kathi mares (1 to 11 parity; yr 1980-92) of the Govt. Horse Breeding Farm, Junagadh (Gujarat) were analysed according to the service dates to see monthly / seasonal variation in gestation length, interfoaling period and foal heat period. The effect of sex of foal and parity was also studied for the said traits. The birth weight and placental weight were analysed only from 1988 to 1992. The year was divided into four seasons according to Kodagali (1970) as Summer (March-May), Monsoon (July-September), Postmonsoon (October-November) and Winter (December-February). The age at first breeding and foaling were estimated considering only the farm bred / born mares. The data were analysed using standard statistical procedures (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

Based on the records of 17 farm born mares, the mean age at first breeding and first foaling were 1713.82±78.42 days and

2106.06±91.80 days, respectively. The overall number of services per pregnancy was 1.62±0.07 based on 214 services required for 132 pregnancies. The fertility rate at foal heat was 53.03%. The proportion of mares concieved in second, third and fourth service was 36.37, 7.58 and 3.03%, respectively, with an overall fertility of 46.97% for nonfoal heat services.

The breeding distribution among high (March-September) and low (October-February) breeding season was 83.2% (104) and 16.8% (21), respectively. For the summer, monsoon,post-monsoon and winter seasons, the breeding frequency was 30.4 (38), 52.8 (66), 8.8 (11) and 8.0 (10 services) percent, respectively. The breeding frequency was considerably low during the months of October to February, then it started incrasing reaching peak in the months of May, June and July. This breeding pattern shows the mares to be seasonal breeder, especially long day breeders, as opined by Hafez (1987).

The gestation period, interfoaling interval and foal heat days did not vary significantly between months of the seasons of the year, and between high and low breeding seasons. However, the gestation period was comparatively longer for summer (331.95±1.6 days) and monsoon (331.06±1.01 days) bred mares than the post-monsoon (327.64±3.08 days) and winter (327.10±2.52 days) bred mares. It was longer (331.38±0.87 days) in high breeding season than the low breeding season (327.38±2.27 days) with an overall mean of 330.71±0.82 days. This is comparatively quite low than the gestation length of 334.14±10.3 to 337.00±10.4 days reported by Tej Singh and Dhinsa (1967), Hadi (1966), Bhyvankumar and Satchidanandam (1987) and Singhvi (1989).

The overall mean interfoaling and foal heat periods were 460.55±16.58 days and 10.21±0.11 days, respectively. The interfoaling interval in Kathi mare was longer than that (545.1±19.5 days) reported by Hadi (1966) in Indian Stabled Horses. Dhinsa (1971), however, reported the interfoaling period of 433 days in Indian mares. The foal heat period is comparatively higher than that (9.66 days) reported by Kodagali (1970). None of these traits was affected by the order of foaling / parity (I to XI).

Out of 125 foals born, 61 (48.8%) were males and 64 (51.2%) were females, the difference being nonsignificant. These findings are comparable to the secondary sex ratio of 46:54 reported by Tej Singh and Dhinsa (1967) and of 49:51 reported by Singh and Raut (1986). The mares carrying colts had nonsignificantly higher gestation length (331.56±1.17 days) than the mares carrying fillies (329.91±1.16 days). Singhvi (1989) observed significantly ($P<0.05$) longer gestation (336.2±0.63 days) in mares carrying male foals than those carrying female foals (333.9±0.75 days). The sex of foal did not affect the interfoaling perior and foal heat days.

The weight of placenta varied nongisnificantly among the months of the year. But the seasons had significant ($P<0.01$) effect on it. Placental weight was higher in summer (4.28±0.18 kg), follwed by monsoon (3.60±0.15 kg), post-monsoon (3.42±0.22 kg) and winter (3.35±0.11 kg). The mean placental weight of mares bred in high breeding season (3.96±0.13 kg) was significantly greater than that of low breeding season (3.37±0.1 kg). Foal's birth weight, though varied nonsignificantly between months and seasons, was higher (39.88±0.74 kg) in high breeding season than in low breeding season (39.36±1.06 kg). These observations on birth weight of foals compared well with those of Singh and Raut (1986) and Singhvi (1979 & 1989) in Indian mares.

Acknowledgement: Authors are grateful to the Head, HRDG, CSIR, New Delhi for the financial assistance given in the form of SRF to Dr. Panchan, and Dr. B.K. Bhavsar, Director of Animal Husbandry, Gujaratstate for his encouragement.

REFERENCES

Bhuvankumar, C.K. and Satchidanandam, V. (1987). Gestational length in Thoroughbreds at Madras, Centaur *3(4):* 127-128.

Dhinsa, H.S. (1971). Studies on some aspects of equine breeding in the army. J. Remount & Vety. Corps, *10(2):* 23-27.

Ginther, O.J. (1979). Cited by Lowis, T.C. and Hyland, J.H. (1991). In Analysis of post-partum fertility in mares on a Throughbred stud in Southern Victoria. Aust. Vet. J., *68(9):* 304-306.

Hadi, M.A. (1966). Studies on eficacy of reproduction in Indian Stabled Horses. Indian Vet. J., *43(8):* 721-723.

Hafez, E.S.E. (1987). Reproduction in Farm Animals. 5th edn, Lea and Febiger, Philadelphia.

Kodagali, S.B. (1970). Reproduction in Kathi horses. Indian Vet. J., *47(5):* 65.

Lay, R.G. (1980). Cited by Lowis and Hyland (1991). In Analysis of ostpartum fertility in mares on a Throughbred stud in Southrn Victoria. Aust. Vet. J., *68(9):* 304-306.

Singh, R.P. and Raut, B. (1986). Study of foaling pattern, birth weight, gestation period and fertility in equines of an equine breeding stud. J. Remount & Vety. Corps., *25(2):* 109-120.

Singhvi, N.M. (1979). A comparative study in equine breeding: Gestation period, foaling time and birthweight. J. Remount & Vety.Corps, *18(1):* 29-35.

Singhvi, N.M. (1987). Gestation in equines and factors affecting it. J. Remount & Vety. Corps, *26(1):* 6-11.

Singhvi, N.M. (1989). Parturition in equines - A comparative note. J. Remount & Vety. Corps, *28(2):* 102-104.

Snedecor, G.W. and Cochran, W.G. (1967). Statistical Methods. 6th edn, IBH Publishing House, Calcutta.

Tej Singh and Dhinsa, H.S. (1967). Reproduction in equines. J. Remount Vety. Corps., *6(4):* 24-33.

IJAR. 16(1), 1995; 50-52

A Method to Induce Chronic Reduction in Uterine Blood Flow in Pregnant Goats

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ABSTRACT

There are numerous pathophysiological conditions under which uteroplacental blood flow is reduced. The best documented examples of chronic reduction in uteroplacental blood flow during pregnancy occur in uterine torsion (Roberts, 1971), eclampsia, twinning, reduced placental size, chronic maternal hypoxia, uterine ischemia, heart disease and diabetes mellitus (Desesso, 1987). To have a comprehensive information on the effect of reduced uterine blood flow in the mother as well as in the fetus it is essential to have a standard reproduceble experimental method. In the present work a method using umbilical cord clamps was used successfully to produce chronic reductions in uterine blood flow for a period of ten days.

——x——x——x——

MATERIALS AND METHODS

Six healthy non descript pregnant does of 4-5 months gestation, weighing about 15 to 20 Kgs were utilized for this study. Pregnancy was confirmed using radiography. The animals were kept under observation for a period of one week. During this priod and subsequently during the period of experimentation the animals were fed with adequate amounts of concentrate, hay and water.

OPERATIVE PROCEDURE

Goat Vascular Anatomy

Unlike most other species the internal iliac arteries of the goat do not arise from the external iliacs but a common internal iliac artery is formed following the bifurcation of the external iliac arteries. This artery divides rather abruptly into the right and left internal iliac arteries each of which in turn divide into the middle uterine and umbilical arteries. The middle uterine artery was considered to be the idealplace to put the clamp for reducing uterine blood flow in pregnancy.

SURGICAL IMPLANTATION OF THE CLAMP

The pregnant goats were fasted for 18 hours and water was withheld for 12 hours prior to surgery. The lower abdominal area was prepared for the midventral incision.

Anaesthesia was induced by a combination of Ketamine (KETASET, Fort Dodge Laboratories, Inc., IOWA) and Xylazine (XYLAXIN, Indian Immunologicals, India). Xylazine was administered intravenously at the rate of 0.22 mg / kg body weight. Ten minutes later Ketamine was administered at the rate of 11mg / kg body weight intravenously (Kumar and Thurmon, 1979).

A mid ventral incision to the length of 4-5" inches extending from the udder to the umbilicus was made after the animal was placed on its dorsal recummbency. The uterus was exteriorised through the laprotomy incision and covered with

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* Part of M.V. Sc., Thesis of the first author submitted to Tamil Nadu Veterinary and Animal Sciences University, Madras.

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a normal saline soaked gauze. The visceral peritoneum over the middle uterine artery was bluntly separated from the broad ligament and the umbilical cord clamps of Denmark make commonly used in new born babies were applied to induce partial occlusion of middle uterine artery. The extent of occlusion was assessed by feeling the pulsation of middle uterine artery. The middle uterine artery of the contralateral horn was also occluded in a similar manner (Fig. 1).

Abdominal muscles along with the peritoneum were closed with continuous sutures using chromic catgut number 'O' and reinforced with another set of sutures. The skin incision was closed by interrupted Halstead sutures using silk (Oehme and Prier, 1980).

In all the operated animals antibiotic coverage with Ampicillin at the rate of 1G was given intramuscularly along with analgesics and intravenous fluid for a period of 5 days. Skin sutures were removed on the seventh day.

During the pre and post treatment period animals were observed for any change in clinical and behavioural signs. On the tenth day or whenever signs of impending abortion or toxemia were observed the animals were sacrificed and the abdoman was opened to confirm the position of the clamps.

RESULTS AND DISCUSSION

Reductions in uterine blood flow for a period of 10 days resulted in significant changes. Abortions and premature delivery were observed in three of the experimental animals. In all the animals the clamps were observed to be undisturbed from the site of first application during pregnancy.

Vascular occlusion is a tool that has been used extensively by investigators to alter the haemodynamics in several vascular beds. Uterine blood flow in pregnant animals has been reduced experimentally by ligation (Greiss *et al.* 1972), Snug fitting bands (Hodgkinson *et al.* 1967), Embolization using microspheres (Creasy *et al.* 1972 and Clapp *et al.* 1980), Vascular occluders (Clark *et al.* 1982) and Clamps (Hooper *et al.* 1990). Most of the above methods were irreversible and excessive reductions in uterine blood flow resulted in severe fetal hypoxia and significant perinatal loss. Since the initial work by Goldblatt and co-workers, a number of alterations have been made in the Goldblatt renal artery clamp. These modifications work well for adjusting renal blood flow, but due to the location of the middle uterine artery these types of unstable mechanisms would be inadequate for regulating uterine blood flow.

The clamp used in the present work allows for both acute and chronic reduction in uterine blood flow in pregnant animals. The clamps remain stable, however, external adjustments are not possible. This method allows reductions in uterine blood flow and alterations in the fetal environment that will facilitate the investigation of the effects of acute and/or chronic fetal hypoxia. The method was simple, effective and reproduceable.

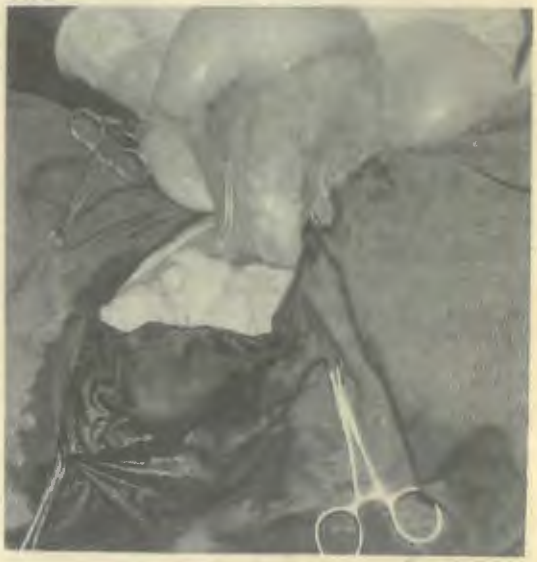

Fig. 1

REFERENCES

Clapp, J.F., Hazel, H. Szeto, Rodney Larrow, Jean Lewitt and Leon L. Mann, 1980. Umbilical blood flow response to embolization of uterine circulation. Am. J. Obstet. Gyencol., *138:* 60-67.

Clark, K.E., Mark Durnwald and J.E. Austin, 1982. A model for studying chronic reduction inuterine blood flow in pregnant sheep. Am. J. Physiol., *242:* H297-H301.

Creasy, R.K., C.T. Barret, M.De. Swict, K.V. Kahanpaa and A.M. Rudolph, 1972. Experimental intra uterine growth retardation in the sheep. Am. J. Obstet. Gynecol., *112:* 566-573.

Desesso, J.M., 1987. Maternal factors in developmental toxicity. Teratogeneis andCarcinog Mutagen, *7:* 225-240.

Greiss, F.C., Jr., S.G. Anderson and L.C. King, 1972. Uterine vascular bed. Effects of acute hyozia. Am. J. Obstet. Gynecol., *133:* 1057-1064.

Hodgkinson, C.P., A. Hodari and F.M. Bumpus, 1967. Experimental hypertensive disease of pregnancy. Obstet. Gyencol., *30:* 371.

Hooper, S.D., C.L. Coulter, J.M. Deayton, R. Harding and G.D. Thorburn, 1990. Fetal endocrine responses to prolonged hypoxemia in sheep. Am. J. Physiol., *259:* R703-708.

Kumar, A., and J.C. Thurmo, 1979. A mixture of Ketamine and Xylazine anesthesia in goats. The Phillipines J. Vet. Med., *18:* 148-153.

Oehme, P.W. and J.E. Prier, 1980. Text book of large animal surgery. 2nd Ed. Wilkinds Co., Baltimore, P.412.

Roberts, S.J., 1971. Veterinary obstetrics and genital diseases. 2nd Ed. Ithaca, New York.

IJAR 16(1), 1995; 53-56

Comparative Efficacy of Different Dilutors for Preservation of Patanwadi Ram Semen at Refrigeration Temperature

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ABSTRACT

The good quality semen ejaculates (90) obtained from 5 adult rams of Patanwadi breed were preserved at 5° under split-sample technique using 3 extenders viz., tris-citric acid-fructose-yolk (TCFY), egg yolk-citrate-glucose (EYCG) and egg yolk-phosphate (EYP). The dilution rate was adjusted at 1:10 (approx 250 million sperm/ml) and samples stored at 5°c till 72 hrs. The percentages of motile, live and abnormal sperm, acrosomal damage and the fructose content of semen were evaluated at 24 hourly intervals. The influence of rams, dilutors, storage intervals and storage x dilutor interaction was significant ($p<0.01$) for all the traits. A steady decline in sperm motility and live sperm percent with proportionate rise in abnormal sperm and acrosomal defects was noted throughout the storage period. The fructose utilization was significantly faster during the first 24 hr of storage, particularly in TCFY diluent, compared with the subsequent storage intervals. The overall efficacy of EYCG was significantly inferior than the other two diluents and TCFY appeared to be the best one for prolonged refrigeration of ram semen.

——x——x——x——x——

The use of artificial insemination in sheep has lagged behind as compared to its use in bovines because of the lack of a satisfactory method of preserving the fertilizing ability of ram sperm for a sufficiently long time (Dauzier *et al.*, 1954). Utilization of chilled semen is still preferred in AI of sheep mainly for want of good freezability and fertility of frozen semen (Nema, 1994). Many extenders have been tested for their efficacy towards improving the preservability as well as freezability of ram semen, yet the goal has not been achieved. Hence, this study was aimed to examine the sutability of 3 diluents for preservation of ram semen at refrigeration temperature.

MATERIALS AND METHODS

This study was undertaken during March to December 1992 on semen of 5 adult Patanwadi rams, ages 16-41 months. The rams were managed indentically under AICRP on sheep breeding at GAU, Sardar Krushingar (Gujarat). Semen was collected weekly in artificial vagina using a male as a teaser. However, for the present study, only 90 fortnightly collected ejaculates (18/ram) were utilized. Soon after evaluation, the samples were split - diluted 1:3 at 23°C in 3 standard extenders viz., tris - citric acid - fructose - yolk (TCFY), egg yolk - citrate - glucose (EYCG) and egg yolk - phosphate (EYP, with 50% yolk). After 5 min, the final dilution was adjusted to 1:10 (approx 250 million sperm/m1) and the semen tubes were transferred to a refrigerator for storage at 5°±1°. The percentages of motile, live and abnormal sperm, acrosomal defects (Watson, 1975) and fructose levels (Mann, 1964) were assessed at 0, 24, 48 and 72 hours of storages. The percent values were arcsin transformed and the data were analysed satistiscally using a 3 factor factorial RBD.

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RESULTS AND DISCUSSION

Motile and live sperm per cent:

The percentages of motile and live sperm atozoa were observed to vary significantly ($P<0.01$) due to rams, dilutors, storage intervals and storage x dilutor interaction, but the ram x dilutor and ram x storage interactions were not significant. The overall values of both the traits were significantly greater for split - samples diluted and preserved in TCFY and EYP diluents as compared to EYCG (Table 1). Although the levels of motile and live sperm at 0 hr were at per between three diluents, the deterioration was much faster in EYCG (17-21%) than in other two diluents (9-13%) even within 24 hr of storage at 5°C, and this trend was further maintained till 72 hrs of storage but at a reduced rate of decline. Sustenance of around 50 - 55% motility and vitality of sperm was observed till 48 hrs in EYCG, and for over 72 hrs in TCFY and EYP diluents. This indicated that the later two dilutors were superior over EYCG for refrigeration storage of ram semen (Table 1).

The mean values and the trends of findings observed in our study compared well with the earlier reports of Abdelhakeam *et al.* (1978). Nicolov *et al.* (1983), Saxena and Tripathi (1984) and Gokcen *et al.* (1990). However, Kharche (1981) recorded better motility and viability of ram sperms in EYCG than in phosphate diluent, which is in contrary to the present findings. Further, El-Gaafary *et al.* (1987) reported much lower values of live sperm % as 75, 63, 53 and 35 at 0,24,48 and 72 hrs of preservation of ram semen in TFY diluent. This was attributed to the difference in breed/initial semen quality and the lack of citricacid in that diluent. Since addition of citric acid to the diluent improved preservability of ram semen (Lafluf *et al.* 1990). The consistently prolonged sustenance of motility and vitality of ram sperm in TCFY diluent might be due to better buffering capcity of tris and its ready penetrance to the sperm cells for intracellular buffering. Similarly, EYP buffer also appeared congenial for survival of ram sperm at 5°C.

Acrosomal defects and abnormal sperm per cent:

The overall mean abnormalities of 6.50±0.63 and 6.71±0.26% noted on dilution (0 hr) in the sperm and acrosomes of ram semen increased highly significantly to the levels of 17.18±0.66 and 31.84±1.29%, respectively, at 72 hrs of storage. Similarly, the pooled values of both the traits were significantly ($P<0.01$) lower (9.59±2.28 and 17.33±4.75%) for the split - samples preserved in TCFY diluent than those in EYCG diluent (11.89±3.15 and 20.49±5.76%), the EYP being intermediate in its efficacy towards preserving sperm morphology and acrosome integrity at refrigeration temperature. The changing trend of these traits was exactly inverse to that of motility and vitality of sperm (Table 1). The percentages of defective acrosomes and abnormal sperm increased at a much faster rate on storage in EYCG as compared to other two diluents. The sperm and acrosome morphology was preserved within acceptable limit till 48 hrs in EYCG, 60 hrs in EYP and upto 72 hrs in TCFY diluents.

These findings of sperm and/or acrosome morphology coincided well with the reports of watson (1975), Skolovskaya *et al.* (1981), Gokcen *et al.* (1990) and Lafluf *et al.* (1990) on ram semen. Saxena and Tripathi (1984), however, did not find significant variation between EYC and EYP (Russian) diluents in respect of morphology of Nali ram sperm. Although the values in Russian diluent were comparable with our observations in EYP. The values of sperm abnormalities and acrosomal defects recorded in ram semen by Nicolov *et al.* (1983) on dilution and after various intervals of preservation at 5°C were, however, much higher than the present findings. This might be due to difference in breed and intial quality of semen tested. Comparatively higher acrosomal damage noted in EYP diluent in relation to motility and vitality of sperm in our study could be due to inclusion of greater amount of egg yolk (50%) in it, as has been opined by Jones and Martin (1973). In the present study, the semen was considered suitable for use in AI till 60 - 72 hrs as the proportion of sperm with intact acrosome, apart from motility/vitality, was much higer (71.51, 66.21

and 67.93% in TCFY, EYCG and EYP diluents, respectively). Skolovskaya *et al.* (1981) also opined that the preserved ram semen was suitable for insemination only if 30% sperm had a normal acrosome.

Fructose utilization:

The amount of fructose metabolised by spermatozoa at 5°C storage was highly significantly influenced by the rams, dilutors, storage intervals and even storage x dilutor interaction. Significantly greater amount of absolute fructose content estimated in TCFY diluent compared to other two diluents was due to its inherent chemical composition. The trend of fructose levels noted between diluents and betwen storage intervals, suggested that the ram spermatozoa utilized fructose as its metabolite at a faster rate in initial stage compared to later intervals at 5°C, and also in TCFY -a chemically most ideal buffering medium, compared to EYP and the least in EYCG buffer (Table 1). The overall actual amount of fructose metabolized by ram sperm during the 1st, 2nd end 3rd 24 hourly interval was 96.2, 62.2 and 46.9 mg%, respectively. The values for TCFY, EYCG and EYP diluents were 77.1, 58.7 and 70.6 mg%. The actual rate of fructose utilization was significantly faster at all intervals in TCFY diluent, poorest in EYCG and intermediate in EYP diluent. The sperm metabolic activity was reduced to almost half by 3rd day (48-72 hrs) of storage compared to 1st day (0-24 hrs) in all 3 diluents. The consistently higher rate of fructose utilization in TCFY diluent proved its efficient buffering capacity in preserving sperm vitality at a much greater level for a long period. Our findings agreed to the report of Tiwari *et al.* (1977) with regard to the effects of dilutors and storage temperature/time on glycolytic activity of ram spermatozoa.

It is thus concluded that TCFY is the most ideal and efficient diluent for long term refrigeration of Patanwadi ram semen at 5°C, in respect of motility, vitality, morphology and fructolytic activity of sperm, followed by EYP in second order and EYCG appeared poorer in this regards.

Acknowledgements Authors thank Dr. K.N. Vyas, Dean; Drs. M.N. Mansuri and P.U. Gajbhiye, Research Scientists, and Dr. B.N. Suthar, Assistant Professor, Faculty of Veterinary Science, SK Nagar for their co-operation and the facilities provided. The first author is grateful to GAU, Sardar Krushinagar for the award of Jr. Res. Fellowship for his master's study.

Table 1. Mean (SE) motility, viability, morphology and metabolic (fructolytic) activity of ram spermatozoa as influenced by various dilutors and preservation (5°C) periods.

Dilutor/ Storage time	Seminal traits				
	Motility %	Live sperm %	Abnormal sperm %	Acrosomal defects %	Fructose mg %
TCFY	65.67±4.96^{a}	72.29±5.95^{a}	9.59±2.28^{a}	17.33±4.73^{a}	1051.78±9.97^{a}
EYCG	56.25±8.25^{b}	63.08 80^{ab}	11.89±3.15^{b}	20.49±5.76^{b}	296.53±8.92^{b}
EYP	64.42±4.99^{a}	71.76±6.41^{a}	10.58±2.68^{ab}	19.76±5.56^{b}	328.74±10.7^{b}
Overall	62.03±4.23	69.53±5.61	10.38±2.87	19.27±4.54	558.35±9.48
00 hrs	77.17±2.19^{A}	87.20±1.92^{A}	4.54±0.63^{A}	6.71±0.36^{A}	674.81±19.6^{A}
24 hrs	65.44±2.46^{B}	71.90±2.33^{B}	8.55±0.66^{B}	15.00±0.64^{B}	576.66±21.3^{B}
48hrs	57.06±2.46^{C}	62.69±2.32^{C}	12.51±0.79^{C}	23.41±1.11^{C}	514.40±20.8^{C}
72 hrs	48.33±2.50^{D}	54.32±2.15^{D}	17.18±0.66^{D}	31.80±1.27^{D}	467.53±19.9^{D}

Means bearing different superscripts in each column differed significantly ($P<0.05$) between dilutors or storage intervals.

REFERENCES

Abdelhakeam, A., Tony, S.M., Yassen, A.M. and E1-Alamy, M.A. (1978). Ram sperm motility aged in glucose and tris buffered extenders at 5°C. Alexandria J. Agri. Res., *26*:301-308 (vide ABA, *47*:3675).

Dauzier, L., Thibault, C. and Winteberger, S. (1954). Preservation of ram semen after dilution and maintenance of its fertily ability. Anim. Breed. Abstr., *23*:292.

El-Gaafary, M.N.S.A., Chamberlin, A.G. and Axford, R.F.E. (1987). Survival rate and fertility of chilled ram semen supplemented with prostaglandin F_2 alpha. Anim. Breed. Abstr., *55* :2196.

Gokcen, H., Soulu, M.K., Tumen, H. and Iglaz, B. (1990). Effects of various additives on the spermatozoai properities and survival time of ram ejaculates diluted with different diluents. Veteriner Fakultesi Dergisi, Uldag Univ, *9-10*:155-161.

Jones, R.C. and Martin, I.C.A. (1973). The effects of dilution, egg yolk and cooling to 5°C on the ultrastructure, of ram spermatozoa. J. Repord. Fert., *35* :311-320.

Kharche, K.G. (1981). Effect of certain dilutors on the viability of ram spermatozoa on preservation at 4-5°C. Indian J. Anim. Repord., *1* :110.

Lafluf, O., Chiossino, M., cresci, A. and Rodriguez, M.H. (1990). Effect of semen diluent pH on sperm motility and fertility of ram semen. Anim. Breed. Abstr., *60* :5114.

Mann, T. (1964) "The Biochemistry of semen and the male Reproductive Tract" John Wiley and Sons, New York.

Nema, S.P. (1994) "Cryopreservation and Associated Biophysical Biochemical Changes in Patanwadi Ram Semen. Ph.D. Thesis, GAU, S.K. Nagar.

Nicolov, I., Radev, G. and Manolov, I. (1983). A comparison of the effects of some semen diluents on the quality and fertilizing ability of fresh and stored ram semen, Anim. Breed. Abstr., *53*:2189.

Saxena, V.B. and Tripathi, S.S. (1984). Preservation of ram semen at 3-5°C. Indian J. Anim. Sci., *54* :813-815.

Skolovskaya, I., Ovidis, R., True, V.B. and Tag, T. (1981). The Significance of acrosome in semen evaluation. Anim. Breed. Abstr., *50* :484.

Tiwari, S.B., Srivastava, A.K. and Sahni, K.L. (1977). Some metabolic changes in ram semen stored in milk diluent. Indian Vet. J., *54* : 111.

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

IJAR. 16(1), 1995; 57-59

Effects of glycerol and equilibration time on the post thaw motility of spermatozoa of buck in maltose egg yolk glycerol extender.

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ABSTRACT

A study was conducted to see the effects of three equilibration periods (1.3 and 6 hours), three glycerol levels (5, 6 and 7 percent) and freezing on the revival rate of motile spermatozoa of buck in maltose egg yolk glycerol extender.

Fifteen pooled ejaculates from 6 Beetal bucks of Goat Research Station, Assam Agricultural University were used for the study.

The highest percentage (82.04±1.21) of motile spermatozoa was recorded after 1 hour of equilibration period with 7 percent glycerol level. However, after freezing the highest percentage (48.79±0.66) of motile sperm could be recorded after 3 hours of equilibration period at 7 percent glycerol level.

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Literature on the freezing of buck semen is meagre. Different equilibration periods and glycerol levels have been tried during freezing of buck semen with varying results in aboard (Westhuysen, 1978) and in India (Sahni and Roy, 1972: Deka, 1984; Sinha *et al.* 1986). But different workers claimed superiority of different equilibration periods and different glycerol levels. The present investigation was undertaken to find out a suitable period of equilibration and a glycerol level for the freezing of buck semen.

MATERIALS AND METHODS

Fifteen pooled ejaculates from 6 Beetal bucks of Goat Research Station, Burnihat, Assam Agricultural University were used for this study. The semen was collected once in a week with the help of artificial vegina. Three different equilibration periods (1, 3 and 6 hours) and three different glycerol levels (5, 6 and 7 percent) were used to observe the molitity during glycerolization, equilibration and after freezing.

Statistical analysis was made as per Snedecor and Cochran (1968).

RESULTS AND DISCUSSION

The mean percentage of motile spermatozoa at different levels of glycerolization, equilibration and after freezing along with the statistical analysis are presented in Table - I.

The mean percentage of motile spermatozoa recorded at fresh semen was comparable to the findings of Deka (1984)

Deka (1984) observed that the percentage of motile sperm before freezing was not significantly affected by equilibration periods but after freezing it was significantly higher. Other workers also did not observe significant effect of equilibration period on post thaw sperm motility in buck semen (Sahni and Roy, 1972). Several workers agreed that sperm motility in frozen ram semen was higher with 7 percent glycerol level (Feredean and Bragaru, 1963 and Boureanu and Negoita, 1971).

Mortimer *et al.* (1976) observed that 7 percent glycerol was superior to 10-11 percent

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glycerol in egg yolk citrate glycerol extender. Patt and Nath (1969) also recorded that 7 percent glycerol maintained a higher post thaw motility. Roussel *et al.* (1964) and Saxena *et al.* (1979) also obtained best results with 7 percent glycerol in freezing of ram semen whereas Salaman and Ritar (1982) obtained best result with 4 percent glycerol level. It was observed that the mean percentage of motile sperm were affected by the percentage of glycerol levels.

The mean percentage of motile spermatozoa was found to vary significantly ($P<0.01$) at various hours of equilibration periods and after freezing. Similar observations were also made by Deka (1984) and Choudhury (1985) in buck semen and in ram semen by Colas (1975) with different dilutors.

The maximum percentage of motile spermatozoa could be recorded at 1 hour of equilibration and it was found to decrease from 1 hour to 6 hours. This was in agreement with Gilbert and Almquist (1978). Kuznekov and Kuprizanov (1959) obtained higher percentage of conception rate from the ram semen equilibrated at 4 to 6 hours. Lightfoot and Salaman (1969) and Conzalez (1976) also obtained best results at 1 hour equilibration period. Patt and Nath (1969) observed that 6 hours of equilibration period was more determintal than 1 hour equilibration in freezing of ram semen. The same result was observed in the present study in buck semen.

On the contrary, Roussel *et al.* (1964) recorded higher post thaw sperm motility in bovine semen, equilibrated with 7 percent glycerol level for 12 hours than 6, 8 or 18 hours and Saxena *et al.* (1979) obtained best result at 10 to 12 hours of equilibration period. Saroff and Mixner (1955) recorded a progressive increase for post thaw motility when the equilibration time was increased from 2 to 8 hours. Westhuysen(1978) observed an increase in percentage of post thaw motility (1 - 2%) at 120 minutes of equilibration period.

The diversity of the opinion of different workers could be attributed to the difference in composition of the extenders, equilibration time or methods of freezing and preservation of the forzen semen.

Table 1: Percentage of motile spermatozoa (Mean±SE*) at different levels of glycerlization, equilibration and after freezing in maltose egg yolk glcerol extender.

Stages	Mobile Spermatozoa		
	5% Glycerol	6% Glycerol	7% Glucerol
Fresh Semen	86.27±1.42	86.27±1.42	86.27±1.42
Glycerolization	82.68±0.94	84.16±1.17	83.85±1.17
Equilibration:			
1 hour	80.05±0.74	82.04±1.21	81.54±1.18
3. hours	74.58±1.17	77.35±1.41	79.52±1.20
6 hours	69.57±0.96	70.92±1.67	71.59±1.46
After freezing:			
1 hour	41.99±1.41	43.28±1.05	45.79±1.18
3 hours	45.99±0.66	47.09±0.81	48.79±0.66
6 hours	37.25±1.23	39.91±1.88	40.33±1.63

* 15 observations.

REFERENCES

Boureanu, A. and Negoita, V. (1971). Freezing of ram semen in ampoules to - 196°C. Revta Zooteh. Med. Vet. *21 :* 33-40. (Anim. Breed Abstr. *40:* 605).

Choudhury, A.H. (1985). Effect of extenders and post freezing preservation on quality of frozen bucksemen. M.V.Sc. Thesis, Assam Agricultural University, Guwahati - 22.

Colas, G. (1975). Effect of initial freezing temperature, addition of glycerol and dilution on survival and fertilizing ability of deep frozen ram semen. J. Reprod. Fert. *42:* 277-285.

Deka, B.C. (1984). Effect of extenders and processing procedures on quality of frozen buck semen. Ph.D. Thesis, Andhra Pradesh Agricultural University, Rajendra Nagar, Hyderabad.

Feredean, T. and Bragaru, F. (1963). The preservation of ram semen by freezing to - 79°C. Luer. Sti. Inst. Cerc. Zootech. *21 :* 357-372 (Anim. Breed. Abstr. *33:* 1377)

Gilbert, G.R. and Almquist, J.O. (1978). Effect of processing procedures on post thaw acrosomal retention and motility of bovine spermatozoa packaged in 0.2 ml. straw at room temperature. J. Anim. Sci. *46:* 225-231.

Jones, R.C. (1969). Influence of diluents and processing times after ejaculation on the survival of deep frozen ram spermatozoa. Aust. J. Biol. Sci. *22:* 995-1004.

Kujnecov, M.P. and Kuprianova, L.P. (1959. Preserving ram semen at a temperature of - 79°C. Trud Vsesojuz nauc. issled. Inst. Zivotn. *23 :* 171-190. (Anim. Breed Abstr. *31-29:* 1517.

Lightfoot, R.J. and Salaman, S. (1969). Freezing of ram spermatozoa by pellet method. II. The effect of method of ditection, dilution rate, glycerol concentration, duration of storage of ram spermatozoa, Aust, J. Biol. Sci. *22 :* 1547 - 60, 61-72. Sydeny, Australia. (Anim. Breed. Abstr. *38:* 2680).

Mortimer, R.G.; Berndtson, W.E.; Ennen, B.D. and Pickett, B.W. (1976). Influence of glycerol concentration and freezing rate on post thaw motility of bovine spermatozoa incontinental straws. J. Dairy Sci. *59:* 2134-2137.

Patt, J.A. and Nath, J. (1969). Effects of diluents, equilibration time and freezing rates on the storage of ram semen, Cryobiology. *5 :* 385 - 392. (Anim. Breed. Abstr. *38:* 528).

Roussel, J.D., Kellgren, H.C. and Patrick, T.E. (1964). Bovine semen frozen in kiquid nitrogen vaour. J. Dairy Sci. *44:* 1403-1406.

Sahni, K.L. and Roy, A. (1972). A note on seasonal variation in the occurrence of abnormal spermatozoa in different breeds of sheep and goat under tropical conditions. Indian J. Anim. Sci. *42:* 501-504.

Salaman, S. and Ritar, A.J. (1982). Deep freezing of Angora goat semen : Effects of diluent compositionand method and rate of dilution on survival of spermatozoa. Aust. J. Biol. Sci. *35:* 295-303.

Saroff, J. and Mixner, J.P. (1955). The relationship of egg yolk and glycerol equilibration time to survival of bull spermatozoa after low temperature freezing. J. Dairy Sci. *38:* 292-297.

Saxena, A.K.; RAizada, B.C. and Pandey, M.D. (1979). Investigations on deep freezing of ram semen. Vet. Res. Bull. *2:* 136-141.

Sinha, S.N.; Singh, B.K. and Sinha, A.K. (1986). Post thaw motility of frozen sperm of bucks of different Breeds. Abstr. Res. Paper, VIth National Cong. Anim. Reprd. Nov.28-30, *1986:* 98 Guwhat Assam..

Snedecor, G.W. and Cochran, W.G. (1968). Stetistical Methods. 6th edn. Oxford and IBH Publishing Co., Bombay, New Delhi, Calcutta.

Westhuysen, J.M. Van der. (1978). Observations on the deep freezing of Angora goat semen. South African J. Anim. Sci. 8 : 111-113. (Anim. Breed. Abstr. *47:* 5548).

IJAR. 16(1), 1995; 60-62

Effect of Oxytocin on Seminal Attributes in Crossbred Bulls

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ABSTRACT

Effect of oxytocin on seminal attributes was studied on two crossbred bulls. 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 the interval of 15 minutes per day, twice a week.

Phase I included normal semen evaluation while during phase II and III, first ejaculate was collected five minutes after the injection of 5 IU and 10 IU oxytocin, respectively. In all, 12 ejaculates were collected during each experimental phase to study the seminal attributes.

Comparison of seminal attributes within phases revealed significantly higher total sperm concentration ($P<0.01$) in Phase II (896.85±68.36 millions / ml) as compared to Phase I (404.03±29.21 millions / ml) and Phase III (596.90±58.63 millions / ml). Head abnormalities were significantly higher ($P<0.05$) in Phase I (4.66±0.42%) and Phase II (5.04±0.65%) than in Phase II (3.45±0.28%). Differences between rest of the macroscopic and microscopic seminal attributes were non-significant between the phases. This studyindicated injection of 5 IU oxytocin ressulted in significantlyhigher total sperm concentration was compared to 10 IU oxytocin.

——x——x——x——

The higher volume of semen coupled with higher concentration of spermatozoa is of prime importance in breding bulls to meet the increasing demand of large number of Artificial Insemination doses. The contractile mechanism involved in sperm transfer in male is partly regulated by oxytocin. Oxytocin causes an increase in gonadotropin secretion which in turn stimulates spermatogenesis (Melin, 1965). It causes effective sperm transport in the genital ducts after exogenous administration (Fjllstrom *et al.* (1968). In the present study, attempts have been made to exploit the bulls which were selected for the breeding purpose by using exogenous oxytocin in two different doses.

MATERIALS AND METHODS

Two crossbred bulls having good sexual behaviour and androgenically non detectable abnormality were selected for this study. Total experimental period of nine weeks was divided into three equal phases as follows:

Phase-I : Normal semen evaluation.

Phase-II : Semen evaluation after intramuscular injection of 15 IU oxytocin.

Phase-III : Semen evaluation after intramuscular injection of 10 IU oxytocin.

During all these three phases semen evaluation was carried out by taking two ejaculates at the interval of 15 minutes per day, twice a week. In Phases II and III first ejaculate was collected five minutes after intramuscular injection of 5 and 10 IU oxytocin, respectively. In all, 12 ejaculates were collected during each phase from each bull and semen was evaluated for its various attributes.

The data were analysed as per the Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The observations made on macroscopic and microscopic seminal attributes during different phases are presented in Table-1.

A) Macroscopic Characteristics

It is revealed from the Table-1 that the average volume of semen in Phase I, II and III was 2.86±0.23, 3.31±0.21 andk 3.52±0.32 ml, respectively. There was non-significant increase in volume of semen in Phase II and III as compared to Phase I, which confirm the views of Bereznev (1963), Milovanov *et al.* (1964), Gorohov *et al.* (1971) and Ibrahim (1968).

No information was available on colour, consistency, density and pH of semen after injecting oxytocin for comparison with the present findings.

B) Microscopic Characteristics

There was gradual increase in the mass activity and initial motility after injecting 5 and 10 IU oxytocin but the differences were non-significant. This is in agreement with the reports of Bereznev (1963) and Ibrahim (1988).

The average total sprm concentrations in Phase I, II and III were 404.03±29.21, 896.85±68.36 and 595.00±58.63 millions / ml, respectively. There was significant increase in the sperm concentration in Phase II and III as compared to Phase I which is in agreement with the findings of Milovanol *et al.* (1964), Gorohov *et al.* (1971), Berndston and Igboeli (1988) and Abreu *et al.* (1993). There were non significant differences in percentage of dead spermatozoa and total abnormal spermatozoa after injecting 5 and 10 IU oxytocin. No similar observations were available for comparison.

C) Comparison of Seminal Attributes between Phases

The perusal of Table-1 reveals that there was non-significant increase in volume, mass activity and initial motility of spermatozoa in Phase II and Phase III than Phase I. However, there was significant ($P<0.01$) increase Phase I and Phase III. Non-significant differences between phases were observed regarding percentages of dead and total abnormal spermatozoa.

It is concluded from the peresent study that significant increase in total sperm concentration could be achieved with injection of oxytocin prior to semen collection. Injection of 5 IU oxytocin had significantly higher total sperm concentration as compared to 10 IU oxytocin.

REFERENCES

Abreu, J.J.; Leite Ribeiro, A.C.C. and Melo, M.I.V. (1993). Effect of oxytocin treatment and message of the cauda epididymis in association with electroejaculation, on ejaculate volume and sperm concentration of bull semen. Anim. Breed. Abstr., *60 (6):* 467.

Berezenev, A.P. (1963). The effect of oxytocin and neurotropic preparation on bull semen. Zivotnovodstovo, *25 (4):* 68-70.

Berndston, W.E. and Igboeli, G. (1988). Spermatogenesis, sperm output and seminal quality of Holstein bulls electroejaculated after administration of oxytocin. J. Reprod. Fert., *82 (2):* 467-475.

Fjllstrom, D.; Kihlstrom, J.E. and Melin, P. (1968). The effect of synthetic oxytocin upon seminal characteristics and sexual behaviour inmale rabbits. J. Reprod. Fert. *17:* 207-209.

Gorohov, L.N.; Konovalov, N.G.; Kovalj, V.A. and Feremova, M.N. (1971). The action of oxytocin on semenproduction in bulls. Anim. Bred. Abstr., *39:* 4602.

Ibrahim, M.A.R. (1988). Influence of oxytocin and prostaglandin on semencharacteristics and process of ejaculation in buffalo bulls. Acta Veterinaria Hungarica, *36 (1-2):* 3-10.

Melin, P. (1965). Indication of stimulant action of oxytocin upon spermatogenesis in rabbits. Anim. Breed. Abstr., *33:* 291.

Milovanov, V.K.; Bereznev, A.P. and Gorohov, L.N. (1964). The effect of oxytocin on reproductive system of male livestock. Anim. Breed. Abstr., *32 (4):* 683.

Snedecor, G.W. and Cochran, W.G. (1967). STatistical Methods, Iowa State University Press, Ames, Iowa, U.S.A.

Table 1: Comparison of seminal attributes in crossbred bulls between Normal and Oxytocin treated phases (n=12).

Seminal Attributes	Phase-I (Normal)	Phase-II (5 IU Oxytocin)	Phase-III (10 IU Oxytocin)
A. Macroscopic characteristics			
1. Volume (ml)	2.86±	3.31±	3.52±
	0.20	0.21	0.31
2. Colour	---	Yellowish white	---
3. Consistency	---	Thin to Thick	---
4. Density	---	D to DDDD	00000
5. pH	6.5±	6.62±	6.66±
	0.00	0.05	0.08
B. Microscopic characteristics			
6. Mass activity	3.03±	3.37±	3.16±
(+ to ++++)	0.13	0.10	0.30
7. Initial motility (+1 to +5)	3.67± 0.11	3.72± 0.20	3.28± 0.24
8. Total sperm	404.03^{a}±	896.85^{b}±	596.90^{c}±
concentration	29.21	68.36	58.63
(millions / ml)			
9. Dead spermatozoa (%)	12.24± 1.34	10.70± 1.01	11.19± 0.79
10. Abnormal spermatozoa			
Head	5.04^{a}±	4.66^{a}±	3.45^{b}±
Abnormalities (%)	0.65	0.42	0.28
Mid-piece Abnormalities (%)	$0.37^{\pm}$ 0.16	0.49^{ab}± 0.50	1.03^{b}± 0.17
Tail	7.83±	5.78±	6.08±
Abnormalities (%)	0.97	0.75	0.30
Total Abnormalities (%)	13.24± 1.41	10.95± 0.98	10.62± 0.48

The means with common superscript in the same row do not differ significantly.

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Seminal Plasma IgG Levels of Holstein Friesian, Jersey and crossbred bulls

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ABSTRACT

Seminal plasma IgG concentrations in relation to Physical characteristics of semen *viz.,* ejaculate volume (ml) sperm concentration million / ml, total sperm concentration / ejaculate, total protein, albumin, globulin, AG ratio and creatinine in Holstein Friesian (n=7) Jersey (n=9) and crossbred (n=6) normospermic and fertile breeding bulls were studied. Significant breed differences for seminal plasma IgG concentrations were not observed. In Holstein Friesian bulls, IgG was significantly ($p<0.05$) correlated with seminal plasma creatinine. In Jersey bulls the IgG concentration was significantly ($p<0.05$) correlated with total sperm concentration / ejaculate. No significant correlation was observed between igG concentration and other parameters studied in three breed gorups.

——x——x——x——

Seminal plasma immunoglobulin levels in different breeds of cattle bulls from temperate region (Bier *et al.* (1978; Suri *et al.* 1986). have been reported.

The present study was taken up to establish the physiological levels of seminal plasma IgG in relation to some physical characteristics of semen and seminal plasma protein profiles of normospermic and fertile Holstein Friesian, Jersey and crossbred bulls.

MATERIALS AND METHODS

Twenty two healthy normospermic fertile breeding bulls ranging in age from 2 to 8 years maintained at Konkan Development Corporation, Aarey Milk Colony, Bombay were used for the study. These bulls were divided into three groups *viz.,* Group I Holstein Friesian, (n=7) Group II Jersey (n=9) and Group III crossbred (n=6) bulls. The crossbred bulls were of varying genetic inheritance of Bos indicus x Bos taurus. All the bulls were maintained under uniform standard nutritional and managemental conditions.

Semen samples were collected twice a week by artificial vagina. A total of 86 semen samples were collected for the present study. Various physical characteristics *viz.,* ejaculate volume (ml) sperm concentration million / ml, total sperm concentration million / ejaculate were studied immediately by using standard methods. The samples were transported to the laboratory in ice. centrifuged at 2000 rpm for 15 min,clear seminal plasma was separated and stored at -20° until used for quantification of IgG. Seminal plasma total protein, albumin, globulin and creatinine were quantified by autoanalyser (550 Express Random Chemistry Analyser, Gillford Systems, U.S.A.). Seminal plasma IgG levels were quantified by single Radial immunodiffusion (SRID) method according to Fahey and Mckelvey (1965). The effect of breed and age of bulls on seminal plasma IgG levels were computed by analysis of variance one way classification and correlation coefficients according to Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Significant breed differences for seminal plasma IgG levels were not observed (Table 1). The mean values of seminal plasma IgG in different breeds observed in the present study were higher than the values reported by Mach and Pahud (1971) by Suri *et al.* (1986) in bulls from temperate climate. This could be due

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to the influence of higher intensity of infection in tropical climate on systemic and local immune defence mecahnisms of the bulls as compared with the bulls of temperate climate (Curtain 1971). No significant variation in IgG concentration between bulls was observed in any of the breed group in the present study.

To study the relationship of various physical characteristics of semen and plasma protein profiles with IgG, the correlation coefficients were calculated, and the results of correlation coefficients among various parameters are presented in Table-2. In Holstein Friesian bulls IgG concentration was significantly correlated with creatinine ($p<0.05$). In Jersey bulls the IgG concentration was significantly correlated with total sperm concentration / ejaculate ($p<0.05$). No significant correlation was observed between IgG and other parameters studied in various breed groups. The results of the present study establish the physiological levels of seminal plasma IgG in Holstein Friesian, Jersey and crossbred bulls from the tropical climate.

The effect of age on seminal plasma IgG levels was studied by dividing the bulls into three age groups *viz.,* 6 bulls of < 3 years, 7 bulls of 3 to 5 years and 9 bulls of > 5 years of age irrespective of their breed (Table-1). Differences in seminal plasma IgG concentrations in different age groups was not significant. These results are in accordance with the results of Suri *et al.* (1986).

Seminal plasma immunoglobulins partly originate from blood by transudation across the epithelium of the genital tract (Rumke, 1974) and partly are synthesised by various male reproductive organs (Rumke, 1974; Foster *et al.* 1988). The class specific immunophysiological role of seminal plasma immunoglobulins in local immunity against various venereal diseases in different breeds of bulls is not clear.

Acknowledgement: The authors are grateful to Dr. R.N. Samant, Chief Veterinary Officer and Dr. S.P. Patil of Konkan Development Corporation for the supply of semen samples, to Dr. M.D. Chauhan, Dean, Bombay Veterinary College, for the facilities given for the present study. The financial grant of Indian Council of Agricultural Research, New Delhi is acknowledged gratefully.

Table 1. Mean ± SEM and range values of IgG concentration in seminal plasma of breeding cattle bulls in relation to breed and age groups.

Group	n	IgG (mg / ml)
Holstein Friesian N = 33	7	0.560±0.019 (0.398-0.832)
Jersey N = 35	9	0.559±0.044 (0.340-0.992)
Crossbred N = 18	6	0.613±0.037 (0.406-0.822)
2 to 3 yrs N = 16	6	0.643±0.053 (0.340-0.719)
3 to 5 yrs N = 28	7	0.530±0.021 (0.406-0.832)
5+ yrs N = 42	9	0.562±0.029 (0.398-0.895)

n = number of bulls
N = number of samples analysed.

Table 2. Cofficient of correlation between igG and various parameters of semen and seminal plasma protein profiles.

Parameters	Holstein Friesian	Jersey	Crossbred
Total Protein	0.258	0.057	-0.764
Albumin	0.524	0.019	-0.053
Globulin	0.601	0.065	-0.797
A/G	-0.045	-0.090	0.299
Creatinine	0.836*	-0.095	-0.383
Ejaculate volume	0.050	0.613	-0.722
Sperm conc./ml	0.109	0.589	0.404
Sperm conc./eja	0.342	0.796*	-0.557

* Significant at $p<0.05$

REFERENCES

Bier, P.J., Hall, C.E.; Dunean, J.R., and Winter, A.J. (1978) Measurement of immunoglobulins in reproductive tract fluids of bulls. Vet. Bulletin. *48:* Abstr. 1830.

Curtain, C.C. (1971) The influence of environment upon serum immunoglobulin levels in cattle. Brit. Vet. J. *127:*442-450.

Fahey, J. and Mckelvey, E.M. (1965) Quantitative determination of Serum immunoglobulins in antibody agar plates. J. Immunol. *94:*84-90.

Foster, R.A.; Ladds, P.W.; Hoffman, D.; and Husband, A.J. (1988). Immunoglobulins and immunoglobulin containing cells in reproductive tract of normal rams. Aust.Vet. J. *65:*16-20.

Mach, J.P. and Pahud, J.J. (1971) Secretory IgA : A major Immunoglobulin in most bovine external secretions J. Immunol. *106:*552-563.

Rumke, P.H. (1974). The origin of immunoglobulins in semen. Clin. Exp. Immunol. *17:*287-297.

Snedecor, G.W. and Cochran, W.G. (1967) **"Statistical Methods"** 6th Ed. Iowa State UniversityPress USA.

Suri, A.K., Gurin, B., Humbolt, P. and Thibier, M. (1986). Effect of infection of the genital tract on the concentration of IgG and albumin in bull serum and semen. Vet. Immunol. Immunopathol *13:*273-278.

IJAR 16(1), 1995; 66-67

A note on successful superovulation and collection of embryos from infertile donor cow

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The degree of success in multiple ovulation and embryo transfer technology is determined to a large extent by the effective management programme of infertile donor cows. The donor cows are classified infertile due to a variety of reproductive abnormalities. One of such abnormality is due to cystic ovary. Cystic ovarian condition is characterised by one or more anovulatory follicular cysts in one or both the ovary persisting for about a week or more with aberrations in sexual behaviour (Bierschwal *et al.*, 1975; Roberts, 1982).

The possible causes of spontaneously occuring ovarian cysts are an excess of follicle stimulating hormone resulting in overstimulating follicular development, subnormal availability of pituitary leutenising hormone (LH) to induce ovulation (Erb *et al.*, 1971) and a partial failure of the mechanism controlling release of pituitary LH (Erb *et al.*, 1973). The present study was accordingly undertaken on the successful superovulation and collection of embryos from the infertile donor cow.

One of the donor cow from the crossbred herd of embryo transfer technology project at IVRI, Izatnagar developed spontaneous ovarian cyst and the animal was showing intense sexual desire and developed male behavioural characteristic such as mounting on other cows. Gynaeco-clinical examination of genital tract revealed enlarged and spherical right ovary with many cystic follicles, swollen vulva, pink colour mucous membrane, open cervical os and good uterine tone.

The cow was treated with a single intramuscular injection of 1500 IU human chorionic gonadotrophin (Chorulon-Intervet, International, Holland) at standing estrus. On day 9 of the estrous cycle, the cow was palpated per rectally for confirmation of corpus luteum (CL) which was present in the right ovary. The cow was superovulated with a single intramuscular injection of pregnant mare's serum gonadotrophin (Folligon - Intervet International, Holland) on day 10 of estrous cycle. The estrus was induced with two injection of reduced does of luprostiol (3.75 mg) administered 48 hrs after PMSG injection through intra vulvo submucosal (IVSM) route, ipsilateral to the corpus luteum at 12 hr interval. The cow exhibited estrus at 48 hr after first injection of luprostiol and was inseminated with liquid semen for 3 times at 12 hr interval after standing estrus. The cow was also treated with 1500 IU of Chorulon at standing estrus to ensure ovulation. Embryos were collected on day 7 by flushing each uterine horn with about 500 ml of modified Dulbecco's phosphate Buffered Saline (D-PBS) supplemented with 0.1% bovine serum albumin (BSA) using 18 or 20 French gauge foley catheter (Bard, New Jersey). The isolated embryos were transferred to holding media (D-PBS with 0.4% BSA) and evaluated morphologically as described by Curtis (1990).

The superovulatory response in terms of number of corpora lutea, unovulated follicles and embryos were 5,4 and 3 respectively. Out of three embryos, two were of good quality compact morulae and one early blastocyst. These embryos were showed further development when cultured in D-PBS supplemented with 0.4% BSA.

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Post superovulatory estrus was induced using IVSM does of luprostiol (3.75 mg each side), cow exhibited post superovulatory estrus at 120 hrs of luprostiol injection. Gynaecolclinical examination of the cow was performed at regular interval to detect any pathological condition. The cow was inseminated at standing estrus with the liquid semen after establishment of the normal estrous cycle and the pregnancy was confirmed at 90 days.

It may be inferred from the above mentioned facts that infertile cow could also be superovulated to get maximum number of viable embryos.

REFERENCES

Bierschwal, C.J.; Garverick, H.A.; Martin, C.E.; Youanguest, R.S.; Cantley, T.C. and Brown, M.D. (1975). Clinical response of dairy cows with ovarian cyst to GnRH. Journal of Animal Sciences, *41*:1660-65.

Curtis, L. John (1990). Cattle embryo transfer procedure. An instructional manual for the rancher, dairymen, AI technicians, animal scientist and veterinarian. pp. 119-26.

Erb, R.E.; Surve, A.H.; Callahan, C.J.; Randel, R.D. and Garverick, H.A. (1971). Reproductive steroids in bovine. VII. Changes post partum. Journal of Animal Science, *33*:1060-71.

Erb. R.E.; Monk, E.L.; Callahan, C.J. andMollett, T.A. (1973). Endocrinology of induced ovarian follicular cyst. Journal of Animal Sciences, *17*:310.

Robert, S.J. (1982). Veterinary Obstetrics and Genital Diseases. CBS Publishers and Distributors, New Delhi, India.

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Fluid Recovery Rate in Superovulated Buffaloes

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The multiple ovulation and embryo transfer technology is of recent origin in buffaloes. Non-surgical flushing of buffaloes using two or three way catheters, the recovered flushing medium was ranging from 74.00 to 79.00 per cent (Sophon *et al.* 1989).

The present study was done to assess the superovulatory response to pFSH and factors influencing the fluid recovery rate in buffaloes.

Nine Murrah buffaloes with a palpable corpus luteum were injected Luprostiol 15 mg (Prosolvin, Intervet, Holland) and monitored for the onset of oestrus using a companion bull. Day zero was taken as Day of oestrus.

From Day 11 to Day 15 of oestrus cycle, control buffaloes (n = 4) received 2 ml saline solution b.i.d. and the treated buffaloes (n = 5) received 1.80 mg of pFSH (Folltropin, Vetrepharm, London) intramuscularly (i.m.) b.i.d. for five days (total 18 mg). On Day 14, all the buffaloes received two doses of Luprostiol 15 mg i.m. at 12 h interval.

Uterine flushing was performed on Day zero Day 6 of oestrous cycle in control and Day 6 of oestrous cycle in pFSH treated buffaloes, with Dulbecco's phosphate buffer saline (DPBS; Gibco Lab, U.S.A) medium, using two way (German-Rusch) catheter. The cuff of catheter was inflated with 10 to 15 ml of air. Each horn was flushed at a time with 20 ml of medium (total 400 ml per horn) and fluid collected in 1000 ml measuring cylinder. The data was analysed by Student's *'t'* test (Snedecor and Cochran, 1967).

There was significantly ($P<0.05$) more fluid recovery during follicular phase (Day zero, 96.56±1.16 per cent) compared to luteal phase (Day 6, 91.50±1.64 per cent) in control animals, which clearly indicates that the luteal phase or presence of corpora lutea reduced the amount of fluid recovered.

On Day 6, the fluid recovery rate in control and pFSH treated buffaloes was 91.50±1.64 and 84.40±1.50 per cent respectively. This indicates that with the increase in number of corpora lutea, there was decline in fluid recovered. This is similar to reports of Sophon *et al.* (1989). Further the fluid recovery was higher compared to the report by Sophon *et al.* (1989) in buffaloes.

The loss of fluid in superovulated buffaloes may be due to technical problems in flushing like inadequate bulb pressure which permits leakage past the bulb (Mc Kelvey *et al.* 1986). There could be escape of flushing medium from uterotubal junction while flushing the uterine horn or better absorptive capacity of endometrium. The endocrine milieu like high progesterone concentration and the reduced uterine tonicity and other physical changes in the reproductive tract could have contributed to decreased fluid recovery rate.

Acknowledgements: The authors are grateful to Director P.A. Sarma, Dr. V.K. Arora and Dr. N. Pratap of Central Frozen Semen Production and Training Institute, Hessarghatta, Bangalore 560 088 for their co-operation and encouragement.

REFERENCES

Mckelvey, W.A.C., Robinson, J.J., Aitken, R.P. and Robertson, I.S., 1986, Repeated recoveries of embryos from ewes by laparoscopy. Theriogenology, *25*:855-856.

Snedecor, G.W. and Cochren, W.G., 1967, *Statistical Methods,* Oxford and IBH, Publishing Co., Ltd., New Delhi.

Sophon, S., Parnpai, R. and Kamonpatana, M., 1989, Multiple ovulation leading to embryo transfer in buffaloes; using PMSG and FSH under oestrus regulation by norgestomet. Buffalo. J., *1*:85-98.

IJAR. 16(1), 1995; 69-70

Gonadotrophin Releasing Hormone Therapy in Infertile Cows and Buffaloes

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Episodic release of gonadotrophic hormones and preovulatory endocrine surge are the important perioestrual events determining the pregnancy rates. Insufficient, asynchronous release or absence of a preovulatory endocrine surge of gonadotrophines might produce a variety of functional reproductive disorders leading to infertility. Gonadotrophin releasing hormone (GnRH) has the properties of inducing a gonadotrophin surge which mimics to a preovulatory endocrine surge (Aminu Deen *et al,* 1991). The elaborate review of literature showed that indiscriminate use of GnRH to improve pregnancy rates in cows being inseminated have not brought encouraging results (Aminu Deen, 1991), but it is felt that the established cases of infertile animals exhibiting a mature follicle and are apparently having disturbances of ovulation process are likely to be benefited with this therapy. With this view, treatment with GnRH was attempted in four infertile cows and buffaloes apparently having problems with folliculogenesis and ovulation, and are presented below.

Case History

Four infertile animals were treated with gonadorelin* (100 μ) (a-c) or buserelin acetate** (100 μg) (d) intramuscularly. History and clinical findings of these animals are as follows:

a) A noncycling crossbred cow exhibited cystic ovaries in its repeated consequent examinations.

b) A nymphomaniac cow had a large cyst in one ovary.

c) A crossbred cow had a history of failure of conception despite being treated previously with all sorts of antibiotics for uterine infection revealed a cystic follicle on one of its ovaries. This cystic follicle persisted in consequent examinations for 3 to 5 days.

d) An infertile buffalo with failure of conception over a period of 1½ years and had taken atleast 20 inseminations was found to exhibit poor symptoms of estrous as revealed by extraordinary poor tonicity, edema and coiling of uterine horns in repeated examinations on consequent estrous cycles.

Post Treatment Response

Normal estrous cycle ensued 7-11 days after the treatment in anoestrus and nymphomaniac cows (a and b) with abolition of symptoms of nymphomania. These animals were inseminated on induced oestrous and subsequently confirmed to be pregnant. Cow c manifested luteinization of cystic follicle after treatment and conceived with first insemination in post treatment estrus. Buffalo also manifested potent symptoms of estrous after treatment and required 2 services to settle.

A sort of preovulatory endocrine surge induced with GnRH turns off the follicular metabolic pathways of a mature, maturing or cystic follicle leading to changes in the follicle culminating into its ovulation or luteinization (Brown *et al,* 1982 and Hernandez-Ledezma, 1982). A corpus luteum or luteinized follicle thus

*Cystorelin, SANOFI
** Receptal, Hoechst

formed secretes progesterone, which normalizes the reproductive events and induce fertile estrous. The only limitation with this therapy seems to be that the immature or small follicles do not respond to the induced gonadotrophin surge. As seen in above presented cases, that administration of GnRH in infertile cows at appropriate time when mature or large sized follicle / cystic follicle was present on the ovary, leads to favourable response.

Administration of GnRH at an appropriate maturity stage of ovarian follicle, in four infertile cows and buffaloes with ovulatory disturbances improved their fertility and all of them were confirmed pregnant.

REFERENCES

Aminu Deen (1991): Effect of Gonadotrophin releasing hormone on postpartum reproductive efficiency of crossbred cows - Clinical and Endocrine aspects. PhD thesis, Haryana Agril. Univ. Hisar, June 1991.

Aminu Deen, Khar, S.K., Khurana, N.K. Galhotra, M.M. and Dixit, V.P. (1991): Effect of GnRH on postpartum ovarian activity. Paper presented in 9th Ann.Conv. of ISSAR, HAU, Hisar, Feb 6-8, 1991.

Brown, E.M., Elmore, R.G., Garverick, H.A. and Kesler, D.J. (1982): Gonadotrophin releasing hormone treatment of dairy cows with ovarian cysts. II. Histology of ovarian cyst walls. Theriogenology *17*(6): 689-696.

Hernandez-Ledezma, J.J., Garverick, H.A., Elmore, R.G., Brown, E.M. and Kesler, D.J. (1982): Gonadotrophin releasing hormone treatment of dairy cows with ovarian cysts. III. Steroids in ovarian follicular fluid and ovarian cyst fluid. Theriogenology *17*(6): 697-707.

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An XX Intersex Calf with Predominant Male External Genitalia

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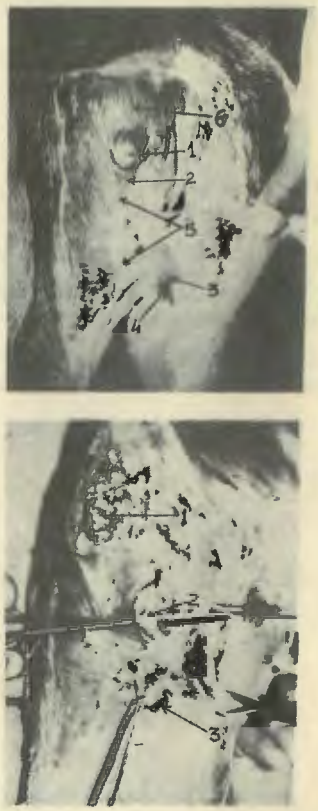
A three months old Jerseycrossbred calf with atresia ani, and both male and female external genitalia was studied clinically and cytogenetically. On examination, the anal orifice was found to be missing and the rectal well ended blindly at its termination. The vulvar opening was present below the blind termination of the rectum. On either side of the vertical vulvar cleft, pigmented smooth vulvar folds were seen. On lateral side of the vulvar opening, genital swelling indicative of the testicular mass was visible as soft fluffy elongated cutaneous folds. The gonadal tissues were palpable within the genital swelling (Fig. 1). The case was operated for the artesia ani and the opening was made for the anus (Fig. 2). The operated genital organs in Fig. 2 shows the penis in the form of a fibromuscular cord passing obliquely, between the thighs, below the perineum. The calf was studied cytogenetically by simplified lymphocyte culture technique (Eldridge, 1982) and the chromosome analysis of 130 metaphases revealed the 60, XX chromosomal complement in all the cells (Fig. 3).

Whereas the examination of external genitalia of the calf indicated the predominance of the male sex organs, cytogenetically the calf was a female having 60, XX chromosomes. The genetic basis of such intersexuality may be a result of the prsence of an autosomal dominant Sxr *(Sex reversed)* gene, which behaves like the H-Y locus of the Y chromosome, having a testes-determining effect (Swanson, 1981). The other possibility of the abnormality may be due to the developmental defect, which may arise due to the action of Mullerian inhibitory factors on the undifferentiated female genital duct. True hermaphrodite was reported in cattle due to the presence of diploid triploid chimerism (Dunn *et al.* (1970). Matejka *et al.* (1989) reported an intersex calf, externally male and having infantile female genital tract, however, cytogenetically 60, XY. No such intersex with predominant male external genitalia, but cytogenetically female (60, XX) could be observed in literature.



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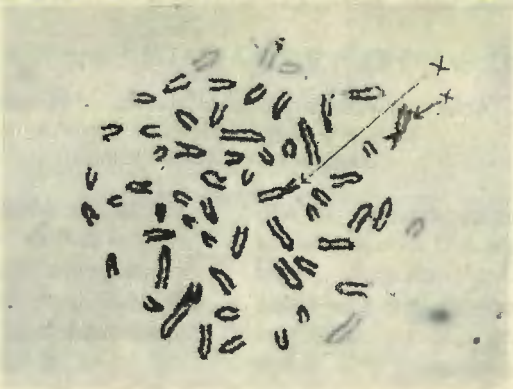

REFERENCES

Dunn, H.O., McEntee, K. and Hansel, '. (1970) : Diploid triploid chimerism in a bovine true hermaphrodite. *Cytogenetics 9:*245.

Eldridge, F.E. (1982) : Bovine lymphocyte methods. *Mammalian Chromosomes Newsletter 23 (4):*176.

Matejka, M., Berland, H.K., Darre, R., Delverdier, M. and Valognes, J. (1989) : XY gonadal dysgenesis in a heifer. *Revue-de-Medecina-Veterinaire 140:*1011.

Swanson, C.P., Merz, T. and Young, W.J. (1981) : Cytogenetics. The Chromosome in Division, Inheritance and Evolution. Prentice-Hall Inc., Englewood Cliff, N.J., U.S.A., p 514.

Legend

Fig. 1

Intersex calf showing congenital abnormalities.
1. Atresia ani 2. Vulvar cleft 3. Preputial orifice 4. Glans penis 5. Perinium 6. Testicular mass

Fig. 2

Post operative structures of the intersex calf
1. Testicular mass 2. Surgically exposed urethra 3. Exposed glans penis

Fig. 3

Metaphase spread of the intersex calf showing 60, XX chromosomes.

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Clinical Observations On Congenital Malformations In Osmanabadi Goats

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Two clinical cases of malformations in Osmanabadi goats were presented to Veterinary Clinics with history of complete gestation. There was no history of any abnormal kidding in previous gestation in both the cases.

Per vaginal examination in first case revealed full cervical dilatation and rupture of uterine bags but foetal parts were not present in birth canal. A dead male foetus was in transverse presentation and it was removed after obstetrical mutations. The hairless skin layer of the kid was thin and it led to rupture of abdomen during obstetrical manoeuver. Second foetus was also removed with slight assistance and it was a mummified foetus having relatively smaller size. There was absence of eyes, cranial development was incomplete, appearance was of dried chicken mass without hairs (Fig 1). Crown-rump length in the premature foetus was 25 cms. as against 15 cms. in mummy. Whereas, heart girth was 10 cms. in premature foetus as against 6 cms. in mummy.

The absence of haircoat and thinning of skin in the foetus indicated that it was a premature kidding. Serological examination of blood sample from the dam revealed absence of antibodies against brucella which ruled out possibility of involvement of brucella organisms for premature kidding. According to the horizons of development in utero (Hafez, 1987) the age of mummified foetus was estimated to be 60 days while it was 90 days in premature kid.

In multitocus animals mummified foetus will not affect other growing foetuses (Roberts, 1971). In present case there was difference between growth and development of two foetuses delivered. Despite the mummification of foetus, the growth and development of co-twin have continued normally.

In another case a Osmanabadi goat presented to the clinics was examined per vaginally and it was revealed that the cervix was dilatated fully and foetal limbs were present in birth canal. A live male kid was delivered with some assistance. Immediately there was apperance of abdominal viscera through birth canal. Further examination revealed presence of foetus in transverse presentation and a dead monster female foetus was delivered after obstetrical mutations.

The monster kid was having acute convex angulation of vertebral column bringing head and tail in close approximation. There was a open abdominal cavity from ventral side and all the abdominal organs were exposed. The abdominal viscera appeared microscopically normal, the forelimbs were also normal except absence of one dewclaw on right side and the hindlimbs were bend caudally right from hock (Fig 2). The monster was a typical Schistosomus Reflexus as per classification of Arthur *et al,* (1989) with severe form of abdominal hernia associated with skeletal defects.

Fig. 1 Foetal mummification

F:g. 2 Schistosomus reflexus

REFERENCES

Arthur, G.H.; Noaker, D.E. and Pearson, H. (1989) Veterinary reproduction and obstetrics (Theriogenology) 6th Edn. Pub. ELBS London pp: 109.

Hafez, E.S.E. (1987) Reproduction in farm animals. 5th Edn. (Indian), K.M. Varghese Co. Bombay. PP: 247.

Roberts, S.J. (1971) Veterinary obstetrics and genital diseases (Theriogenology), 2nd Edn. CBS Publishers and distributors, New Delhi, India. PP: 173.

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A Case of Dystocia due to Diplopagus monster in a buffalo

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Dystocia due to duplication of cranial end of the foetal body is uncommon and has been reported earlier (Pandit *et al*, 1994). Successful treatment of dystocia due to sternopagus, discephalus (Siamese) twin monster in buffalo is presented.

An emergency call was made at Civil Veterinary Hospital Nawan Shahar (Jalandhar) to attend a case of dystocia in a Nili Ravi buffalo. Per vaginum examination of the dam revealed breech presentation of the foetus. Epidural anaesthesia (15 ml of 2% lignocaine hydrochloride) was given. By repulsibn and traction the hind legs were extended into the pelvis. Two tails were felt and the possibility of two foetuses was ruled out by further examination. The foetus was delivered by correction and forced traction and it was dicephalus, distomus, tetrapus, dibrachius dead female monster (Fig). The postmortem of monster revealed duplication of thoracic and abdominal viscera.

It could be a case of diplopagus conjoined twins. These twins arise from a single ovum and are monozygotic (Roberts, 1970), however they can be dizygotic also (Potter, 1961). Their incidence is one in 100,000 in bovine births (Arthur, 1956 and Hancock, 1954).

Embryonic duplications are malformation due to abnormal duplication of germinal area giving rise to foetuses whose body structures are partially duplicated In this case, there was no history of monstral birth from the last 3 normal parturitions. So, it could be a case of teratogenic monster.

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REFERENCES

Arthur, G.H. (1956). Conjoined and identical twins. Vet. Rec. *68* : 389.

Hancock, J. (1954). Monozygotic twins in cattle in Advance in Genetics. 6, 141, Academic Press Inc. N.Y.C.

Pandit, R.K, Pandey, S.K. and Agrawal, R.G. (1994). A case of dystocia due to diplopagus monster (Craniopagus twin) in a Goat Indian J. Anim. Reprod. 15(1) : 82.

Potter, E.L. (1961) Pathology of foetus and infant 2nd Ed. Year book Med. Publisher, Chicago.

Roberts, S.J. (1970). Veterinary Obstetrics and Genital diseases, 2nd Ed, CBS Publications and Distributors, (India).

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Management of Habitual Abortion in a Bitch

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Incidence of habitual abortion due to endocrine deficiencies in canines is not known (Roberts, 1971). Progesterone deficiency due to poor luteal function might be one of the causes of habitual abortion with nfections or genetic factors being the other (Aurhur *et al.* (1989). Corpora lutea are necessary to maintain the integrity of the preganancy throughout its duration (Sokolowski, 1971). Premature regression of corpus luteum during sixth to seventh week may lead to abortion (Blom, 1968).

Case History and Treatment: A 8 year old Labrador bitch weighing around 18 kg was presented with the history of abortion / premature birth between 45-52 days consecutively for the last four terms. The pups delivered live, died between 1-2 hours after whelping. This time the bitch was mated to a male Labrador. By abdominal ballotment animal was confirmed to be pregnant at 30th day of gestation. Keeping in view the threat of abortion or premature birth the animal was given inj. Hydroxyprogesterone caproate 250 mg i/m on 42nd day of gestation and was repeated a week later at same dose rate. On 60th day the bitch delivered 4 male and 3 female healthy pups.

Incidence of habitual abortion seems to be low because of few reports and 5-25 mg doses of progesterone in oil 2-3 times a week untill the 8th week of pregnancy (Blom, 1968) or progesterone implants (Arbeiter, 1968; Blom, 1968) have also been tried successfully.

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REFERENCES

Arbeiter, K. (1968). Wieh Tierartz. Mschr., *55:*40.

Arthur, G.H., Noakes, D.E. and pearson, H. (1989). Veterinary Reproduction and Obstetrics. 6th Edn. ELBS Pub. London.

Blom, F. (1968). Canine Medicine, Edit. by E.J. Catcott Amer.Vet. Public, Inc., Wheaton, Ill., 445.

Roberts, S.J. (1971). Veterinary Obstetrics and genital diseases (Theriogenology). 2nd Edn. CBS Publication.

Sokolowski, J.H. (1971). J. Anim..Sc., *21:*696.

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Bacteriological Studies of Cervical Secretion of Repeat Breeder Cows

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Efficient dairying and breeding demands that a cow shall give birth to a healthy calf twelve months and be in milk for at least 300 days in a lactation. But the repeat breeding has been recognised as one of the most serious problems affecting the economy of dairy industry. Though the specific causes for the condition can not be pinpointed, the presence of micro-organism in uterus has a significant role in the failure of conception. Indiscriminate use of antibiotic invariably results in the development of resistant bacterial strains complicating the effectiveness of therapeutic measures.

The observation herein reported are based on a study of 76 cross bred cows brought to the college clinics and has repeated at least three times from regularly spaced services. Cervical secretion of repeat breeder cows were collected aseptically in sterilized sheath (Dabas and Maurya, 1988) at the time of estrus.

Each sample was streaked on blood agar plates in order to obtain isolated colonies of bacteria. After inoculation for 24 hours at 37°C, those colonies which appeared to be those of staphylococci were marked and fished out by straight platinum wire and subcultured in nutrient broth and again incubated for 24 hours. The broth cultures were transfered to agar plates in order to get isolated colonies. Colonies other than those of staphylococci that appeared on blood agar plates were picked up. These were purified and identified according to Buchanan and Gibbons, 1974).

In Vitro antibiotics sensitivity/resistance pattern of isolates were carried out against 13 antimicrobial drugs.

Out of 76 cervical samples a total of 58 (76.3%) samples were positive to bacterial infection. The distribution of microflora indicated the highest percentage of Gram positive cocci (60.4%) followed by mixed infections (27.6%) and Gram negative rod (12%).

The majority of cervical samples were found sensitive to Erythromycin (89.6%), Cloxacillin (93.1%), Ampicillin (86.2%), Chloramphenicol (89.6%) Nelidixic acid (94.8%, Nitrofurantoin (87.9%), Co-trimexazole (82.7%). Most of the samples showed resistance against Penicillin, Streptomycin, Oxytetracyclin and tetracyclin.

Intra-uterine infusion alongwith parenteral administration of the most effective drug(s), either single or in combination of two for 4 to 5 days at the time of estrus resulted in conception in 46(79.3%) animals within next two estrous cycles post-treatment.

The present study revealed the presence of micro-organisms that pose a potential threat to normal fertility and are contributory factors to repeat breeding. Unless these organisms are removed, fertility can not be restored. Antibiotic sensitivity testing should invariably be carried out in order to rationalize the treatment and overcome genital tract infection. However, utmost care should be observed in applying these drugs, as any indiscriminate use may lead to a more serious drug resistant bacterial forms and thus be permanent resisdent, of the genital tract.

REFERENCE

Buchanan, R.E. and Gibbons, N.E. (1974. Bergy's Mannual of Determinative Bacteriology. 8th ed. The Williams and Wilkins Co., Baltimore. pp 484-485.

Dabas, Y.P.S. and Maurya, S.M. (1988). A field method for collection of bovine cervical mucus for microbiological studies. *Indian J. Anim. Reprod. 9* :138.

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Influence of Dry Period on Economic Characteristics In Jersey Cows

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The dry period influences economic production of dairy animals. The present study deals with the variation and effect of dry period on economic characteristics in purebred Jersey cows under tropical conditions.

A total of 111 records of purebred Jersey cows imported from Australia and maintained at the Jersy Cattle Farm, Banavasi (A.P.) formed the material for the study. The cows were in para 1 to 5 and the period of study was 6 years (1984-89). The date on dry period (DP) was partitioned at class intervals of 60 days and its effect on lactation milk yield (LMY), Lactation Length (LL), Milk Yield per day of lactation (MYDL), per day of calving interval (MYDC), Service period (SP), Calving interval (CI) and birth weight (BW) was analysed as per Snedecor and Cochran (1967).

The average DP was 132.0±10.2 days. The proportion of cows having DP of <60, 61 to 120, 121 to 180, 181 to 240 and >241 days was 8.1%, 60.4%, 11.7%, 4.5% and 15.3% respectively.

Milk Production: The average LMY was 2419±57.2 kg and that of LL was 317.9±4.2 days. The cows which had longer DP (>241 days) also had higher LMY (2570.7±200.8 kg) and LL (348.3±20.6 days), while those with an optimum DP of 61 to 120 days had LMY of 2438.7±and LL of 312.8±6.2 days. The shorter DP of <60 days was not found to adversely affect either LMY or LL with respective average of 2315.1±155.5 kg and 307.4±12.3 days. Further the effect of DP on both the traits was not satistically significant.

The MYDL varied from 7.07 to 7.81 kg with an average of 7.72±0.1 kg. Cows having DP of 61 to 180 days had higher yields, while the cows with DP of less than 60 days had marginally lesser value than herd average. However, the difference was not satistically significant.

The MYDC varied from 3.8 to 6.4 kg ($P<0.01$) with an average of 5.4 kg. It was highest for the cows with DP of less than 60 days and decreased progressively as DP increased. This is obviously a sequel of progressive increase in the calving interval with the increase of DP.

Service Period: The average SP was 168.8±11.2 days. It was lowest (80.0 days) for the cows with DP of less than 60 days and increased linearly with increase in DP, the maximum being 367.2 days for cows with DP of above 241 days. The difference was significant ($P<0.01$).

Calving Period: The average calving interval was 450.5±30.5 days. The lowest CI was 362.3 days for the cows with DP of less than 60 days and then increased similar to that of SP with significant ($p<0.01$) differences among DP groups. This trend of SP and CI is a reflection of the fact that the factors which were responsible for the longer DP obviously resulting from longer SP might also be responsible for longer SP and longer DP in the subsequent lactations.

Birth Weight: The average BW was 20.8±0.4 kg. It was lowest (19.7±0.9 kg) following a DP of <60 days and highest (23.4±2.8 kg) following DP of 181 to 241 days. However, the differences were not statistically significant.

ACKNOWLEDGEMENTS: The authors thanks are due to Dr.M. Yogi Reddy, Director of Animal Husbandry, Andhra Pradesh, Hyderabad for according permission to publish the data.

REFERENCES

Snedecor, G.W. (1967) and Cochran, W.G. - Statistical Methods Iows State University Press, Ames, USA.

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Distal Mid-Piece Reflex Defect in a Buffalo Bull

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Detailed and rological examination of a normal healthy Surti Buffalo bull aged 11 years (Date of birth : 29.8.84) revealed normal libido with reaction time of 3.5 mts. Accessory glands were normal with palpable lobulations. The scrotal examination revealed torsion of the left testis rotating clockwise by 90° displacing right testis to the left side.

Semenology revealed : Volume of semen 2.74±0.08 ml; initial motility 2.66±0.33; sperm concentration 993±81.50 10^6/ml; live sperm 77.60±2.52%; Abnormal sperms 35.8±1.91%. The predominant sperm abnormality was distal midpiece defect with trapped droplet (DMRD). The DMRD defect was noticed even from the first semen collection made on 13-4-1989 when the bull was about 5 years old. In a period of one year 44 semen collections were made for this study. Based on over all semen score the bull was graded as questionable.

Sequential collection (partial exhaustion test) revealed decline in volume of semen from 2.5 ml to 1.7 ml by fifth collection. Abnormal sperms with DMRD declined from 33 per cent to 10 per cent by fifth collection. Motility, sperm concentration and live sperm percentage did not change by the test. The sodium and potassium concentration in the seminal plasma were observed to be different from the normal value.

The DMRD is characterised by the sperm having a bend in the distal region of the mid-piece in the shape of letter or a shepherds hook with cytoplasmic droplet trapped in the bent portion. Folding or coling of the tail is also noticed. This defect could be due to malformation of outer supporting sheath or dense fibres of the midpiece and tail portion of the sperm. This defect is similar to Dag defect reported by Blom (1966). Johnson and Pederson (1971) further described the Dag defect as coiling of midpiece with traping of distal protoplasmic droplet.

Blom and Welstrup (1976) attributed excess of Zinc as causal factor of Dag defect. Besides many adverse stimuli scrotal insulation was also considered to produce this defect. In the bullunder study the torsion like deformity noticed in scrotum and testis could have altered the thermo regulatory mechanism of testis thus resulting in DMRD sperm defect. The decline in the incidence of sperms with DMRD defect after sequential collection indicate the possibility of epididymal disfuction (Swanson and Boyd, 1961).

Acknowledgement: Thanks are due to Dr. R.K. Shukla, Principal, Veterinary College, Anand for providing facilities; Emeritus Scientist Scheme of ICAR, New Delhi for financial help and Staff of Dept. of Gynaecology of Obstetrics for all the help.

REFERENCES

Blom, E. 1966 A new sterilising and hereditory defect (Dag defect) located in the bull sperm tail Nature *209:*739-740.

Blom, E and Welstrup, C. 1976 Zinc as a possible causal factor in the sterilising sperm tail defect (Dag defect) in Jersey bulls. Nord. Vet. Med. *28:*515-518.

Johnson, H.H. and Pederson, H. 1971 Further observation on the Dag defect of the tail of bull spermatozoa. J. Reprod. Fertil. *26:*77-83.

Swanson, E.W. and Boyd, L.J. 1961 Factors affecting coiled tail spermatozoa in the bull Am. J. Vet. Res. *23:*300-309.