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Microbial agent in infectious reproductive diseases: Control and Application of Biotechnology

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Bovine infertility has been recognised as a serious problem causing direct loss to the herd owners and a serious barrier restricting the genetic progress achieved through various well layedout projects, specially in developing countries. The very fact that some herds function normally in respect of fertility when the others do not, and in some cases fertility has been substantially improved by concentious effort, show that the problem is not one which cannot be alleviated. Identifying infertile animals sufficiently early and presenting them to a veterinarian can help the breeders to reduce losses. Modern laboratory facilities and treatment possibilites remarkably widended the scope of solving problems of infertility. Abortion, stillbirth and the premature birth collectvely termed as Abnormal Termination of Pregnancy (ATP) is the chief cause of annual loss of calf crop in organised dairy herds in the country. As such a study was conducted to determine the various epidemiological features of abortion/ATP in organised herds under the scheme for Investigation into infectious abortion in Livestock in West Bengal sponsored by the Indian Council of Agricultural Research. Various features of Abnormal Termination of Pregnancy (ATP) in 13 organised herds in West Bengal for a period of 4 years are presented.

Overall incidence of ATP was 16.1% of which abortion 9.7%, still birth 4.4% and premature birth was 1.9%. However, out of 680 ATP cases, 60.4% were abortion; still birth and premature birth represented only 27.3% and 12.2% respectively.

Monthwise occurence of ATP observed varied from 11.9% on February to 20.2% in June. Out of the 4 quarters highest 17.3% ATP was observed in the 2nd quarter ie., from April to June and least 14.3% during the 1st quarter i.e. from January to March. Highest number of abortions took place in the month of July while highest number of still birth and premature birth took place in the months of October and May, respectively. ATP occured with nearly equal frequency in 2nd 3rd gestation i.e. 25.1% and 25.7% respectively. Out of 680 ATP cases by normal test it has been found that ATP at 3rd gestation was significant at 5% level in comparison to other gestations.

Overall seroprevalence of brucellosis in organised herds in West Bengal among cattle was 20.5% while the same in the bulls and cows was 14.1% and 21.3%, respectively. Prevalence of brucellosis in buffaloes in organised herds was 19.3% out of 808 tested; 14.8% buffalo bulls positive out of 27 tested and 19.4% she buffaloes were positive out of 781 tested. Prevalence of brucellosis according to herd size revealed that in herds having less than 100 cattle it was 10% and herds having more than 200 cattle it was 26%. Number of cattle in a herd influece managerial efficiency and thereby influence the dissemination of infective agent.

Prof. C.R. Sane oration lecture delivered in Silver Jubilee- XIV annual convention of ISSAR held at Bidar on 14th Nov. 1997.

Comparative prevalence of brucellosis in cattle groups of farms housing only exotic, only indigenous, only crossbreed and indigenous mixed has shown that prevalence was more in farms maintaining only crossbreed. No such earlier studies have been made. Contrary to Polding (1947), the farm maintaining only exotic cows was found to be free from brucellosis. Thus introduction of exotic germ plasm does not seem to have contributed to the high prevalence in crossbred herds; rather presence of infections in the herd coupled with building up of large herd with crossbred cows for milk yield without appropriate managerial input seem to have resulted in high prevalence of brucellosis in herds composed of crossbred cattle.

Prevalence of brucellosis was observed in cattle farm practising either natural service or artificial insemination. In farms practising natural service only 6% of the bulls were positive out of 33 tested and in cows 4.2% out of 623 tested, while in farms practising A.I., 14.9% of the bulls were positive out of 334 tested and 27.4% of cows out of 1352 tested. This is probably because of distribution of a single ejaculation of semen in large number of cows in A.I. Brucella organisms are not continuously excreted in each ejaculation. So in natural service there is lesser chance of infection by a single ejaculation.

Prevalence of brucellosis was also analysed according to the nature of farming ie., farms maintaining only cattle or cattle and buffalo both. Out of 20 farms, 14 maintained only cattle and prevalence was 15.2% serologically. In the remaining 6 farms having cattle and buffalo both, prevalence was 23.2%. So in the latter group prevalence was much higher than the other. Though prevalence of brucellosis among buffalo of organised herd was very close to that of cattle herds, it is interesting to note that prevalence was much higher in the herds maintaining a mixed population of cattle and buffaloes. This needs further study and evaluation.

Incidence of different infections namely Brucellosis, Leptospirosis, Campylobacteriosis an Trichomoniasis was studied in relation to 4 different types of reproductive disordrs namely, ATP., endometritis, repeat breeding and anestrus condition in 795 cows of 36 Development Blocks of 13 districts of West Bengal in rural areas. Prevalence of brucellosis was 10.6%, 0%, 2.4% and 1.4%, respectively in 66, 72, 41 and 284 of above types of cases; 12.5% of leptospirosis in repeatbreeding cases only out of 35, 72, 41 and 12 of the above types of reproductive disorders; 20.2%, 0%, 16.1% and 1.6% of compylabacterosis in 55, 72, 149 and 121 of these types of cases, respectively. Out of 41, 72, 135 and 129 cases, respectively T.fetus was successfully stained and cultured from a repeater cow in rural areas (De *et. al.,* 1983 and 1989).

In a subsequent study on incidence of T. fetus by this author and coworkers, three strains of T. fetus were isolated in culture from preputial washings from 44 breeding bulls in different organised herds in West Bengal. These were of "Belfast" type only. (Mondal *et. al.,* 1983).

In a further studty on Leptosopriosis, spiral bodies morphologically identical to Leptospira organisms were demonstrated in the tissues (Kidney and spleen) of two aborted fetuses in two organised herds and in the issues of guineapigs inoculated with tissue suspension from one of the aborted fetuses by Levaditis silver impregnation techniques (De *et. al.,* 1983). Serological tests of 47 aborted cows against various infections revealed about 21% positive for

brucellosis and about 28% for IBR while only 4.2% was positive for Q fever. The seradiagnosis of brucellosis was confirmed in 12 cows by RBPT and MET. (De *et. al.,* 1989).

The development of colony blot ELISA presents an interesting application of monoclonal antibodies in rapid Brucella identification. Vezcaino and Fernandez (1992) found a coaglutination test using monoclonal antibody highly useful for rapid, simple, specific and low cost of identification of Brucella species. Chand *et. al.,* (1990) used dot-ELISA with a plate ELISA for diagnosing bovine brucellosis using serum samples. Lieu *et. al.,* (1991) in tests with Dot-ELISA, ELISA, R-SAT (Agglutination test) and R-coomb's test proved Dot-ELISA more reliable. ELISA Kit is very useful for diagnosis of brucellosis. Modification of competitive ELISA (C-ELISA) is a reasonable test for differentiating the antibody response of cattle vaccinated with Brucella abortus strain 19 and B.abortus infected cattle (Neilsen *et. al.,* 1995).

Detection by polymerase chain reaction (PCR) for routine diagnosis has been investigated by Fekete *et. al.,* (1990). An examination of field specimens compared with bacteriological examination revealed PCR to have specificity (96%)) and sensitivity (98%). New methods such as restriction fragment length polymorphism (RFLP) and multilocus enzyme electrophoresis could be exploited with markers that can differentiate strains.

Another possible approach is application of embryo transfer technology as method of interrupting the transmission of disease (Hare, 1985). Many vaccines are already in use-strain 19 and the killed 45/20 etc. Adult vaccination produces resistant serum antibodies, interferes with correct interpretation and diagnosis using official Kits. S 19 may produce permanent infection in bulls and vaccination is not recommended.

Merien *et. al.,* (1992) used polymerase chain reaction successfully for detection of Leptosira sp in clinical samples. Hum *et. al.,* (1994) reported ELISA measuring IgA antibodies in vaginal mucus to diagnose bovine venereal compylobacteriosis in 241 herds with infertility and abortion. Bovine placental invasion by T. fetus was readily detected by indirect immunoflourescence employing monoclonal antibodies for T. fetus detection (Burgess and Knoblock, 1989). Rapid detection by nucleic acid probes such as ribosomal RNA that are being applied to some parasites (Waters and McCutcheon, 1990) may also find value in the diagnosis of bovine trichomoniasis.

Engelenburg *et. al.,* (1983) developed a rapid and sensitive PCR, assay for detection of IBR bovine herpes virus type I in bovine serum and in contaminated semen. Pandita and Srivastava (1995) compared Dot-ELISA and Plate ELISA for detection of BHV-I antibodies . Dot-ELISA was found better as a field test as it is easier and more economical to perform. Xia *et. al.,* (1995) observed detection of BHV I in semen, by dot-blot hybridisation, PCR and virus isolation methods.

De and Basu (1967) diagnosed M. bovis from endometritis in a cow in West Bengal. There are many such records. All these may cause reproductive diseases. On the more practical side elimination of semen contaminants should remain to be primary concern. Antibiotics used are getting resistant and list of opportunistic and potential pathogens in semen are increasing. Novel methods to control contaminants such as use of antibodies (monoclonal and

genetically engineered antibodies), anti-idiotype vaccines, and exploitation of natural inhibitors in the semen should be looked at as variable alternatives for supplements to antibiotic treatment. In the next decade there will be increasing number of diagnostic kits for rapid, highly specific identification of infections and toxic agents.

With current trends towards trade harmonisation and emphasis on safety of products there is a need to adopt rational and uniform standards for microbial quality of semen and diagnostic tests to monoitor its quality, maintenance of bulls and also cows (both cattle and buffaloes) free from specific diseases.

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Parthenogenesis in Buffalo (Bubalus bubalis)*

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ABSTRACT

Successful Parthenogenetic buffalo embroys were produced utilizing ethanol and cytochalasin B for activation process. The activation was induced after four different hours of maturation (30, 33, 36 and 39) and the oocytes cultured for 5 h in 5 ug/ml cytochalasin B medium. Among the parthenogenetic embryos thus produced, 30.4 per cent reached the blastocyst stage. At oocyte maturation hour of 39 h, maximum oocytes cleaved (20.3%), whereas maximum oocytes reached the blastocyst stage (6.1%) at a maturation hour of 36 h, and both these rates of development were not different either from each other or from other hours of maturation.

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The eggs of mammals could be induced to develop in the absence of a fertilizing spermatozoon, by various chemical and physical stimuli including calcium-free medium, alcohol, heat and electric shocks. Such parthenogenentic embryos were, of course, all females. They may continue develop apparently normally until mid-gestation, but they had never as yet survived until birth, even when the sigle haploid chromosome set of the egg had been doubled up to give the normal diploid number of chromosomes.

MATERIALS AND METHODS

Buffalo ovaries brought from the slaughter house were aspirated for oocytes and washed thoroughly. The oocytes were subjected to maturation in TCM-199 with 5 per cent FBS and antibiotics in the CO^2 incubator with 5 per cent CO^2 in air at 38.5°C for different hours. Mixed grade oocytes at 30, 33, 36 and 39 h maturation period along with cumulus cells were subjected to activation by treating them with 7 per cent ethanol (Bengal Chemicals, India) in PBSA for 7 minutes. The treated oocytes were then shifted to TCM-199 supplemented with 5 per cent FBS and 5 ug/ml of cytochalasin-B cultured at 38.5°C for 5 h. After 5 h, the activated oocytes were washed and cultured in TCM-199 with 5 per cent FBS in CO^2 incubation in same drops where they were matured. The parthenogenetic cleavage and growth was monitored for 9 days. After every 48 h of culturing, the fresh TCM-199 was introduced into the culturing drops.

RESULTS AND DISCUSSION

At 30, 33, 36 and 39 hours of oocyte maturation time, the oocytes when subjected to alcohol activation resulted into cleavage at the rate of 15.8, 19.1, 18.5 and 20.3 per cent, respectively. The cleavage rates were not different when subjected to statistical analysis even at 5 per cent level of significance. A clear-cut trend of increase/decrease in the per cent oocytes cleaved was not observed in the experiments. In an identical study, recording the cleavage rate of bovine parthenogenetic activation by Fukui et al. (1992) through ethyl alcohol, the authors noticed that the five maturation durations for oocytes (24, 27, 30, 33 and 36 hours) were also not

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significantly different for cleavage between two- to eight-cell stage. (Table 1).

With 7 per cent ethanol and cycloheximide treatment of young bovine oocytes (IVM for 24 h), an activation rate of 46.7 per cent was obtained which was as efficient as results obtained through electric activation (Goto *et. al.,* 1994), whereas we have done our studies with ethanol activation only and not through electric activation. Winger *et. al.,* (1995) reported that at 34h IVM time, 7 per cent ethanol activation followed by incubation with TCM containing cytochalasin, cleavage rate of 53.6 per cent was obtained. These authors reported the rate of blastocyst stage development of 14.2±1.8 per cent, which is very different from our results for blastocyst production.

In the present study, the rate of blastocyst production at 30, 33, 36 and 39 maturation hours on alcohol activation and culturing was 5.3, 5.9, 6.1 and 5.1 per cent, respectively. Between 30 and 36 hours maturation time, it increased from 5.3 to 6.1 per cent and then fell to 5.1 per cent. The differences were not statistically significant.

Fukui (1990), in a similar study recording the cleavage rate and per cent oocytes retaining cumulus cells developing to blastocyst stage reported that blastocyst production rate of 2.8±2.0, 8.8±5.5, 6.6±3.9, 6.7±0.3 and 6.7±3.6 per cent, respectively. The results obtained are very similar to our results where the rate of blastocyst production was inferior ($p<0.01$) at 24 hour maturation time compared to other longer maturation hours of 27, 30, 33 and 36 hours. There was no difference in the blastocyst rate of production during maturation hours of 27, 30, 33 and 36.

In bovine oocytes, Boediono and suzuki (1994) reported a higher blastocyst production rate of 17 per cent when activated by ethanol and cultured with cytochalasin B. In the oocytes which were activated only and not cultured with cytochalasin B, blastocyst production rate was 1 per cent. Winger *et. al.,* (1995) recently reported mean rates of development to blastocyst stage, which is higher than our results even for unselected oocytes. The respective rates of selected and unselected oocytes were 26.8±4.0 and 14.2±1.8percent, respectively.

In a study on parthenogenetic development of buffalo oocytes and activation through electri stimulation, Taneja and Singh (1994) reported that oocytes could be activated by a single electri pulse, however, multiple pulses of high field strength apparently yield a better rate of parthenogenetic development. They further reported that both activation and development appear to be dependent on oocyte age. Their rate of development of oocytes to blastocyst stage were similar to our study for 24 and 28 hour of maturation time and was 4 per cent. This rate was, however, increased significantly to 24 per cent when oocytes were matured for 32 hours.

Aside from these technical considerations, there is the possibility that there are biological constraints placed upon the parthenogenetic development of buffalo pre-implanation embryos that are not in place for bovine embryos. Such constraints might be related to the influence of genomic imprinting upon buffalo early development. The oocyte activation method presented in this report coupled with the successful production of parthenogenetic pre-implantation buffalo embroyo through to the blastocyst stage now afford at least a blue print for preliminary investigation regarding the role of the maternal and paternal genomes during buffalo early development to be pursued.

Table 1. Effect of maturation time on parthenogenetic embryo production

Oocyte maturation (h)	Number of oocytes	Number oocytes cleaved (% of oocytes)	Number oocytes reaching blastocyst (% of oocytes)	Blastocysts per cent (% of cleaved)
30	57	9 (15.8)	3 (5.3)	33.3
33	68	13 (19.1)	4 (5.9)	30.8
36	65	12 (18.5)	4 (6.1)	25.0
39	59	12 (20.3)	3 (5.1)	25.0
Pooled	249	46 (18.5)	14 (5.6)	30.4

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Effect of exogenous administration of $PGF_2\alpha$ at different stages of estrous cycle on onset of estrus and subsequent fertility in crossbred cattle

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ABSTRACT

Effect of administration of $PGF_2\alpha$ at different stages of estrous cycle on onset of estrus and subsequent fertility in crossbred cattle was studied. The results revealed on major differences in estrus interval, percentage of animals in estrus, pregnancy rate and AI per conception due to stage of estrous cycle at which $PGF_2\alpha$ was injected. The overall fertility in $PGF_2\alpha$ treated animals (pooled data) and untreated control was almost similar (93.1 vs. 92.5%) indicating unimpariment of fertility following the administration of $PGF_2\alpha$.

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Exogenous administration of $PGF_2\alpha$ and its synthetic analogues between day 5 and 16 of the estrous cycle causes luteolysis and decrease in serum progesterone in cattle (Louis *et. al.,* 1975). Most of the animals return to estrus within 2 to 4 days following the administration of $PGF_2\alpha$ (Kudlac and Vinkler, 1980). Interval to onset of estrus after $PGF_2\alpha$ injection in cattle has been reported to be affected by the age, breed, season and stage of the estrous cycle (Jaster *et. al.,* 1982; Stevenson *et. al.,* 1984; Graves *et. al.,* 1985). In the present paper effect of stage of estrous cycle at $PGF_2\alpha$ treatment on onset of estrus and subsequent fertility in crossbred cattle is reported.

MATERIALS AND METHODS

The experiment was conducted on crossbred cattle selected from the Institute's dairy herd. Animals were divided into 4 groups viz., group A (n=10, day 6 to 7), group B (n=10, day 8 to 11), group C (n=10, day 12 to 15) and group D (n=12, control).

Animals of groups A, B and C were given single intramuscular injection of $PGF_2\alpha$ (2 ml Prosolvin, Intervet International, Holland) between day 6 to 7, 8 to 11 and 12 to 15 of the estrous cycle, respectively. All the animals of groups A, B and C had palpable corpus luteum in any one of the ovary at the time of $PGF_2\alpha$ injection. In group D animals were not treated with $PGF_2\alpha$ and served as a control. Following the administration of $PGF_2\alpha$, animals were subjected to detection of estrus twice daily with the help of a teaser bull and external estrus symptoms. Animals confirmed in estrus were inseminated with frozen semen. They were subjected to confirmation. Time taken for onset of estrus following $PGF_2\alpha$ administration, pregnancy rate and AI per conception were calculated and presented.

RESULTS AND DISCUSSION

Onset of estrus and subsequent fertility following the administration of $PGF_2\alpha$ given at different stages of estrous cycle is presented in Table 1. Estrus interval following $PGF_2\alpha$ injection was 70.8±10.38 hrs in groups A, B and C, respectively. All animals (100%) in group A and C and 9

(90%) in group B exhibited estrus following the administration of $PGF_2\alpha$. The results indicated on major differences in the estrus interval and percentage of animals in estrus due to the stage of the estrous cycle at which $PGF_2\alpha$ was injected. Some authors had reported difference in the onset of estrus interval after $PGF_2\alpha$ injection due to stage of estrous cycle (King *et. al.,* 1982; Stevenson *et. al.,* 1984) which is in contrast to the observations of the present study. However, the above workers could not precisely explain the reasons for such differences. No difference in serum progesterone concentration and luteal regression due to the stage of the estrous cycle at which $PGF_2\alpha$ was injected have been reported (Stevenson *et. al.,* 1984) which may explain reasons for no difference in the onset of estrus interval among the different treated groups of the present study. Overall results indicated that out of 30 animals treated with $PGF_2\alpha$ between day 6 to 15 of the cycle, 29 (96.6%) were in estrus within 71.02±6.42 hrs. Most of the authors have reported onset of estrus in majority of animals within 2 to 4 days following the administration of $PGF_2\alpha$ (Chauhan *et. al.,* 1982; El-Menoufy and Abdou, 1989) which is in agreement to the observations of the present study.

The results further revealed that stage of estrous cycle at which $PGF_2\alpha$ was injected, did not affect the subsequent fertility. The pooled data on fertility indicated no difference in pregnancy rate and AI per conception between $PGF_2\alpha$ treated and untreated control (93.10 vs. 92.50%; 2.2 vs. 2.4), indicating unimpairment in the fertility after the administration of $PGF_2\alpha$. Encouraging fertility results using $PGF_2\alpha$ has also been reported earlier (Agarwal *et. al.,* 1987) which corroborate the findings of the present study.

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Table 1. Effect of stage of estrous cycle at $PGF_2\alpha$ treatment on onset of estrus and subsequent fertility in crossbred cattle.

	Treatment with $PGF_2\alpha$ on days				Control
	6 to 7 Gr.A	8 to 11 Gr.B	12 to 15 Gr.C	Overall	Gr.D.
Animals treated (n)	10	10	10	30	12
Animals in estrus	10(100%)	9(90%)	10(100%)	29(96.6%)	—
Time taken for onset of estrus after $PGF_2\alpha$ injection	70.8±4.63	66.66±4.26	75.6±10.38	71.02±6.42	—
Pregnancy rate n	10	8	9	27	11
%	100.00	88.8	90.0	93.1	92.5
AI per conception	2.2	2.5	2.1	2.2	2.4

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Comparative efficacy of a low dose of dinoprost or luprostiol for estrous induction in superovulated crossbred cattle.

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ABSTRACT

Thirty six crossbred cows in two groups (n1=n2=18) were induced for estrus (Prior to and during superrovulation) with either 5.0 mg of Dinoprost (Group 1) or 3.0 mg of Luprostiol (Group 2) by IVSM route ipsilateral to the side of CL. Post superovullatory estrus was induced on day 10 in respondent donors only (n1=11,n2=13). The percentage of post superovulation were 88.88,100 & 72.72 for group 1 and 94.44, 100 & 76.92 for group 2, respectively. PG to estrus interval was 73.08±3.59, 39.84±1.49 & 130.50±5.25 hours for group 1 and 66.00±3.27,40.42±1.08 & 124.20±3.61 hours for group 2, respectively. These differences were statistically non-significant (P>0.05). It was concluded that low doses of Dinoprost and Lurprostiol were equally effective for inducing estrus in superovulated crossbred cattle.

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Prostaglandin F2-alpha is routinely administered by intramuscular (I/M) route for induction of estrus in various species including cattle. Reports indicate that a low dose of $PGF_{2}\alpha$ (10 to 25% of I/M dose) by intravulvo-submucosal route can effectively be used for estrus induction (Cordova *et. al.,* 1990; Pawshe *et. al.,* 1991) or other therapeutic purpose (Ono *et. al.,* 1982; Narsimha rao *et. al.,* 1985). In an earlier study from this Lab (Khanna *et. al.,* 1995), estrus was induced in superovulated crossbred cattle administering 3.75 mg of Luprostiol by IVSM route. In the present study, an attempt was made to further reduce the dose (3.0 mg) and also to compare its efficacy with Dinoprost at the same propertionate dose level (5.0 mg).

MATERIALS AND METHODS

A total of 36 crossbred cows (HF, BS, Jersy Crosses with Hariana) having normal reproductive tract and 3-7 years of age were selected for this experiment. The animals were divided into two groups at random (n1=n2=18) and were induced for estrus prior to superovulation by either 5.0mg of Dinoprost (Lutalyse, Upjohn, Belgium) (Group 1) or 3.0mg of Luprostiol (Prosolvin, Infar India Ltd.,) (Group 2) by IVSM route ipsilateral to the side of CL (Khanna *et. al.,* 1995). Estrus was induced during superovulation using same route and doses 48 hours after the initiation of gonadotropin treatment (Super-OV;50 units; 10.5/10.5, 7.5/7.5, 4.5/4.5, 2.5/2.5 units). Post superovulatory estrus was induced on day 10 only in respondents, administering same dose on either side. Estrus was detected twice daily with the help of teasser bull and behavioural signs of estrus. Statistical analysis was done by Micro-32 computer applying chi-square test (Snedecor and Cocharan, 1968).

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RESULTS & DISCUSSION

When estrus was synchronized prior to superovulation, 88.88% animals (16/18) in group 1 (Dinoprost group) and 94.44% animals (17/18) in group 2 (luprostiol group) exhibited estrus 73.08±3.59 and 66.00±3.27 hours after the administration of PG, respectively. A higher percentage of animals came to estrus earlier, in group 2 as compared to group 1 yet, these differences were statistically non significant (P>0.05). An earlier study reported a synchronization rate of 75% in Holstein heifers using Luprostiol in a varied dose rate (1.5 to 7.5 mg). By IVSM route, PG to estrus interval was 61±6 hours (Cordova *et. al.,* 1990). Better results obtained in the present study (Luprostiol group) may be due to difference in dose level (3.0mg) and pleuriparous crossbred cattle used in this experiment.

An earlier report from this lab (Khanna *et. al.,* 1995) has indicated a synchrozation rate of 91.67% with 3.75 mg Luprostiol. The present study further confirms the above findings with a lower dose (3.0 mg). Dinoprost, a naturally occurring $PGF_2\alpha$ has also been used in an earlier study demonstrating a synchronization rate of 81.75% with 10 mg dose in crossbred cattle (Pawshe *et. al.,* 1991). In this study a better synchronization rate (88.88% Dinoprost group) has been obtained using half the dose of compared study (5.0 mg).

When estrus was induced during superovulation, all the animals (100%) in both groups exhibited estrus at 39.84+1.49 and 40.42+1.08 hours after PG injection in group 1 and group 2, respectively. The results in the present study are in accordance with the earlier study (100%;40.60+0.57 hours) where 3.75 mg Luprostiol was administered by IVSM route (Khanna *et. al.,* 1995). PG to estrus interval was almost similar irrespective of the analogue used and also with the earlier report. Similar results have been reported in the literature using $PGF_2\alpha$ or its analogue for induction of superovulatory estrus by intramuscular route (Wubishet *et. al.,* 1986; Agarwal, 1990). A higher percentage of animals in estrus during superovulation might be a resultant of increased estradiol secreted from gonadotropin (exogenous) stimulated multiple follicles (Betteridge, 1977).

When estrus was induced on day 10 post superovulation, 72.72% animals in group 1 and 76.92% animals in group 2 exhibited estrus at 130.50±5.25 and 124.20±3.61 hours, respectively. These findings were simillar (87.5%, 111.07±6.60 hours) to the earlier reported from this lab. (Khanna *et. al.,* 1995). A slightly lower induction rate may be due to the individual variation in the experimental animals. A low estrus response was observed following administration of $PGF_2\alpha$ analogue by intramuscular route (60%; Dinar *et. al.,* 1987) and IVSM route (52.94%; Khanna *et. al.,* 1995) immediately after embryo recovery. Since, extended period of ovulation during superovulatory estrus results in the formation of corpora lutea of different ages, $PGF_2\alpha$ might fail to ensure complete luteolysis of the younger ones (Cooper, 1974). A higher percentage of animals coming to estrus in the present study(and in previous study referred earlier) may be due to the availability of $PGF_2\alpha$ responsive corporalutea which had crossed the unresponsive stage i.e. 4-6 days post ovulation (Cooper, 1974) It was concluded that a low dose of both Dinoprost and Luprostiol by IVSM route is effective for estrus induction in superovulated crossbred cattle.

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Endocrine Profile in Superovulated Crossbred cattle

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ABSTRACT

Daily plasma samples were assayed for estradiol 17B (E2) and progesterone (P4) in 11 crossbred cows superovulated with 40 mg FSH-P (multiple dose) or 2500 IU PMSG (single injection). E2 and P4 levels on day 0 of natural estrus were 9.60 pg/ml and 0.46 ng/ml. E2 at supervolutory estrus was 1.9 times higher than natural day 0 estrus. Peak E2 level was 19.24 and 18.21 pg/ml on day 7 after estrus (day of flushing) in PMSG and FSH.P groups, respectively. The corresponding values for P4 were 0.44 and 0.18 ng/ml at supervoluatory estrus and 13.30 and 3.05 ng/ml on day flushing in PMSG FSH groups. The correlation between number of CL and P4 level on day of flushing in PMSG group (0.30) is lower than FSG-P group (0.32).

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Studies on superovulation in cattle began 40 years ago (Zavadowaski and Eskin, 1939) however, the variability of response in quantity and quality is still large. Since the administration of exogenous gonadotrophin to cattle has a profound effect on their normal endocrine balance and embryo quality (Greve *et. al.,* 1984), the analysis of hormones have led to a better understanding of the causes of variability in superovulation response and thus pave the way for control of quality and quantity of embryos. The present study accordingly was undertaken to get insight in blood plasma hormonal level of crossbred cows after superovulation treatment and their correlation with superovulatory response.

MATERIALS AND METHODS

Eleven crossbred cows superovulated by administering either with 40 mg FSH-P (multiple injection 7/7, 6/6, 5/5, 2/2) or single injection of 2500 IU pregnant mare serum gonadotrophin starting from day 11 of estrous cycle followed by single injection of μg prostaglandin analogue (Carboprost, Upjohn Ltd., Crowley, Sussex, England) 48 hrs later in both the groups. Response to superovulation was measured as number of corpora lutea (CL) and unovulated follicles palpated per rectally 7 days after superovulatory estrus. Animals were bled daily between 6 and 7 a.m. starting from day of estrus (day0) upto 7 days of superovulatory estrus. After collection plasma was separated by spinning at 3000 rpm for 5 min and stored at -20°c till assayed. Concentration of progesterone (P4) was estimated by using a direct solid phase (I^{125}) RIA kit (count A, count TK PG Diagnostic Products Corp, LA, CA, USA) after solvent extraction. The sensitivity was 0.05 ng/ml. The inter and intra assay variations were 9.4 and 6.7% respectively. Estradiol 17β (E2) was assayed by using method of Hall and Sufi (1981) with few modification. The sensitivity was 2.5 pg/ml.

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The inter and intra assay coefficient of variation was 10.4 and 7.5% respectively.

RESULTS AND DISCUSSION

The concentration of E2 on day 0 of natural estrus was 9.60±1.18 pg/ml and was 10.30±1.27 pg/ml on day 11 when superovulation treatment was started. The concentration of E2 started rising within 24 hrs of gonadotrophin treatment and reached a peak value of 18.21±5.36 and 19.24±2.88 pg/ml in FSH-P and PMSG groups, respectively, within 48 hrs of PG injection. A longer interval between PG injection and superovulatory estrus was observed in FSH-P treated cows (46.18±4.81 hrs) as compared with PMSG treated cows (34.80±6.68hrs) although the peak E2 levels were not different. Comparatively early onset of estrus after PG injection in PMSG treated group of animals is also reported by Yadav *et. al.,* (1986) and kweon *et. al.,* (1987). A high level of E2 is involved in initiating LH surge (Scaramuzzi *et. al.,* 1971) early production of estradiol to a high level presumably initiated the LH surge earlier in animals of PMSG group (Henricks *et. al.,* 1973). The correlation between peak E2 and superovulatory response was 0.42 and 0.43 in FSH-P and PMSG groups, respectively.

Post ovulatory increase of E2 was observed in the PMSG treated group. The same was also observed by kweon *et. al.,* (1987) and Agarwal *et. al.,* (1993). Post ovulatory rise of E2 in PMSG group was probably due to post ovulatory follicular growth as a result of longer half life of PMSG (Schams *et. al.,* 1978). In FSH-P group E2 level remained at basal level. The E2 level on day of flushing was 8.38±0.88 in FSH-P group and 7.66±2.11 pg/ml in PMSG group.

The average P4 concentration at natural day 0 estrus was 0.22±0.08 ng/ml. It started rising by day 2 and reached an average concentration of 1.57±0.27 ng/ml on day 11 (mid luteal) when gonadotrophin treatment was started. The P4 concentration increased at a rapid rate following gonadotrophin injection except in two cows which showed decrease in P4 concentration. Decrease in P4 level after gonadotraophin treatment was also reported by Solti *et. al.,* (1978).

The synchronising PGF_2-alpha injection 48 hrs after gonadotrophin injection caused a sharp decline in P4 level to a 0.18±0.04 ng/ml and 0.44±0.21 ng/ml at standing estrus respectively, FSH-P and PMSG treated groups. However, in only one cow the P4 level was above 1 ng/ml at standing estrus. The similar result was reported by Booth *et. al.,* (1975). This may reflect the insufficiency of PGF_2 to cause luteolysis after the process of gonadotrophic stimulation of corpus lutem (Saumande, 1980). However, a part of P4 may also come from the follicle (Thibier and Saumande, 1975).

The increase in P4 level after superovulation occured earlier and to a higher level in PMSG treated group (13.30±4.48 ng/ml) than FSH-P treated group (3.05±2.21 ng/ml). The correlation between number of CL and P4 level on day of flushing on PMSG group (0.30) is lower than in FSH-P group (0.32). Apparently, the higher P4 was secreted by multiple CL, however, unovulated luteinized follicles had also been reported to secrete appreciable amount of progesterone (Booth *et. al.,* 1975).

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Serum Progesterone Profile in Buffaloes treated with CIDR-Device and Combinations

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ABSTRACT

In all 39 non-cyclic buffaloes were assigned to short (8 days) and long (12 days) term therapy either with CIDR impant of proluton depot in combination with prostaglandin and PMSG. In CIDR implanted groups a rapid increase in P4 levels on day 2 with subsequent fluctuating trend on day 6 and 8 was observed. Whereas, in group receiving proluton depot after an initial increse a slow and uniform decline was observed. With short term therapy the P4 level decreased from day 6 to 8. With long term therapy with CIDR a sharp decline was noted while in group with plain CIDR, P4 level was almost constant on day 6 to 12. On day of oestrus basal P4 levels (<0.12 ng/ml) were observed in all groups except in T2 group. Long term P4 priming (12 days) was prefarable to short term (8 days) therapy. However, the latter could produce better results with combination therapy.

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Some evidences exist to show that short term progesterone treatment may not result in the same degree of oestrogenic activity as is associated with long term progestagen regimen. Adequate levels of progesterone or its anologue must be maintained during treatment to suppress oestrus and to maintain satisfactory fertility at the synchronised oestrus. In this context, although the objectives of maintaining average plasma progesterone concentration (PPC) to suppress endogenous luteninsing hormone (LH) release have been achieved the degree of variation in PPC among individual treated animals is not reported. The study of variation in the hormonal levels is expected to reveal mode of changes leading to regular sexual rythm and fertility and the use of appropriate hormone preparation in judicious dose schedule.

MATERIAL AND METHODS

In all 39 healthy, buffaloes were gynaeco-clinically confirmed as anoestrus and those with similar age and parity were assigned to the various experimental groups. (Table 1). CIDR (controlled internal Drug Release) devices are manufactured by Carter Halt Harvey Agricultural Division, Newzealand. The device contains 1.9 gm of micronised U.S.P. grade progesterone. The other drugs used were proluton depot (German Remedies Limited), Folligon (PMSG Intervet) and Lutalyse (Dinoprost Tromethamine - Upjohn Co.) CIDIROL (estradiol benzoate) Capsules.

To study the serum biochemical profile of progesterone (P4), blood was collected on Day 1 (prior to start), Day 2 (Second day) and Day 6 (Mid) Day 10 (PGF2 alpha injection), Day 8/12 (removal of implant) and on day of expression of estrus.

For progesterone estimation from serum samples Radioimmuno Assay was performed by using ready progesterone kits obtained from Diagnostic System

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Laboratories, Webster, Taxas U.S.A. The lower limit of sensitivity of assay system was found to be 0.1 ng standard progesterone and it helped to measure progesterone in 100 ml. Data for above parameters were statically analysed as per Snedecor and Cochran (1967) and presented.

RESULTS AND DISCUSSION

The serum P4 level at various stages with different treatment is shown in Table 2. Most of anoestrus buffaloes showed the initial P4 level below 0.12 ng/ml. The P4 level observed in the present study is in agreement with that reported by Chede et al (1992).

After the insertion of CIDR implant the serum P4 level in group. T1, T4 and T6 showed a rapid increase on day 0 to day 6. Similar rise and fall in P4 level was observed on day 8 or 10 in these groups. The average P4 level of 0.24+0.06 ng/ml observed on day 0 prior to treatment reached to 2.49+0.63 ng/ml on second day. Present observations are in agreement with Rao *et. al.,* (1987).

Dose of Prolution Depot

In group T2 and T3 prolution depot 1000 mg was injected in divided or single dose instead of CIDR implant. The fertility of 66.66 and 33.33% was observed in respective groups. In these groups the initial P4 level showed increase from day 0 to two followed by a slow and uniform decline on day 6.

The details reveal that in group T3 out of 6 cases. 3 showed almost constant P4 level on day 2, 6, 8 or 10 of the collection. The failure to exhibit a comparable progesterone profile at these stages may be attributed to the slow release or absorption or progesterone. Thus low P4 profile further failed to exert a stimulatory inducing effect and fertility in such buffaloes. Gurdial Singh *et. al.,* (1983) reported similar findings in the animals treated with PRID alone.

Short Term Treatment

In group T3 prolution depot 1000 mg was injected as one dose wherein the P4 level was 0.70+0.50 ng/ml on day 6 which after PGF2 alpha injection decreased to 0.28+0.16 ng/ml on day 8. In group T4 CIDR and .OB were implanted, the P4 level was 2.85+1.14 ng/ml on day 6 which declined to 1.03+0.35 ng/ml on day 8. However, in group T5 receiving CIDR implant the P4 level increased to 2.14+0.84 ng/ml on day 8. After implant withdrawl these animals received 500 I.U. PMSG (Folligon) to facilitate folliculogenesis. The fertility of 33.33, 50.00 and 71.42 per cent observed with these treatments indicate the superiority of use of oestradiol benzoate and PMSG over the previous one in sequence. In group T3 probably the P4 profile did not show adequate increase required for supression of LH and FSH release. Progestagen treatments have frequently been used with PMSG to stimulate oestrus and ovulation. Although, treatment with CIDR device alone altered the distribution of intervals from diagnosis to oestrum, a PMSG injection at device removal stimulated behavioural oestrus in larger percentage of animals.

Long Term Treatment

In Buffaloes in group T1 (CIDR AND PG-2 alpha) P4 level reduced from day 10 to 12. In group T2 (prolution depot) the level had already declined on day 10 to cause any appreciable variation on day 12. Whereas in group T6 (only CIDR for

12 days) P4 level was almost constant around 1.69+0.60 ng/ml on day 12. The fertility recorded in the aforesaid groups was 85.71, 66.66 and 71.42 per cent respectively.

In group T1 the synchrony and fertility was 100.00 per cent and associated with the reduction of the P4 level. These observations are in accordance with Smith *et al.*, (1984) with CIDR alone for 12 days (group T6) probably such synchrony could not be achieved as evident from the higher P4 level on day 12 followed by a slight reduction in fertility.

Post therapy progesterone (P4) profile

In the short or long term therapy after removal of the device the plasma progesterone is rapidly inactivated and metabolised causing a controlled drop in P4 leading to follcular maturation. The P4 levels on the day of oestrus were, 0.12 ng/ml in all groups except in T2 where it was estimated to be 0.35+0.02 ng/ml, the average being 0.15+0.03 ng/ml. Present findings are in agreement with Rao *et. al.*, (1987) and McMillan *et. al.*, (1991) who used PRID for 12 days.

The results of the present investigation reveal that the amount of progesterone released by CIDR device represented an adequate level to cause a progesterone priming necessary for increasing follicular recruitment. The associated plasma progesterone concentration values were influenced by the type and duration of the therapy employed. The long term P4 priming (12 days) was preferable to short term (8 days) therapy. However, the latter could produce better results with combinations drugs.

Table 1. Experimental groups with various treatment regiments.

Sr. No.	Group	No.of Buffaloes	Treatment
1.	T1	7	CIDR impant for 12 days and PGF2 alpha 2.5 ml. 1/M on day 10.
2.	T2	6	prolution depot 500 mg on 1st and 7th day and PGF2.
3.	T3	6	Prolution depot 1000 mg on 1st day and PGF2 alpha 2.5 ml/ 1/M/on day 6.
4.	T4	6	CIDR plus Estradiol Benzoate for 8 days plus PGF2 alpha 2.5 ml on day 6.
5.	T5	7	CIDR for 8 days and Folligon (PMSG) 500 I.U. at removal of implant.
6.	T6	7	Only CIDR implant for 12 days (control for treatment)

Table 2. Serum progesterone (ng/ml) levels at various stages and groups of treated buffaloes.

Sr. No.	Group	Before Treatment	Day, 2	Day, 6	Day, 8	Day, 10	Day, 12	Day estrus
1.	T1	0.37 ± 0.25	3.20	0.81 ± 0.09	—	0.80 ± 0.24	0.41 ± 0.12	<0.01
2.	T2	0.20 ± 0.07	2.90 ± 0.69	0.45 ± 0.24	—	<0.12	0.15 ± 0.03	0.35 ± 0.22
3.	T3	<0.12	2.40 ± 1.49	0.70 ± 1.49	0.28 ± 0.16	—	—	<0.12
4.	T4	<0.12	1.90	2.85 ± 1.14	1.03 ± 0.35	—	—	<0.12
5.	T5	0.47 ± 0.33	2.00	0.93 ± 0.21	2.19 ± 0.84	—	—	<0.12
6.	T6	0.17 ± 0.05	1.41 ± 0.35	1.69 ± 0.37	—	—	1.69 ± 0.60	<0.12
7.	Average	0.24 ± 0.06	2.49 ± 0.63	1.03 ± 0.19	1.24 ± 0.39	0.49 ± 0.17	0.62 ± 0.23	0.15 ± 0.03

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Ovarian Response to PMSG and GnRH among Buffaloe Heifers.

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ABSTRACT

Eight non cycling murrah buffalo heifers of 12 month age received synchronizing and superovulatory regimen of PGF_2 alpha(30 mg and 25 mg) and PMSG(Folligon 2500IU) combination. Four heifers (experimental) received (iv) 200ug synthetic GnRH(Fortagyl) while remaining four heifers (control) received (iv)2ml saline solution at induced estrus. The mean interval from PMSG injection to onset on estrus and duration of estrus was respectively 96.00±8.93 hrs and 66.00±6.00 hrs in experimental while 101.00±8.06 and 70.00±15.10 hrs in control group. The number of palpable follicles and ovulation respectively was 7.00±1.00 and 5.50±0.50 in experimental, while 7.5±1.19 and 4.75±0.85 in control group. GnRH injection at induced estrus did not have any influence on total number of ovulation while it caused synchronized ovulation within 12 to 26 hrs of its administration. The ovulation in saline treated control was phased and delayed spreading over 96 hrs.

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It has been observed that superovulatory response of pregnant mare serum gonadotrophin (PMSG) is approximately double in heifer than cow(Voss *et. al.*, 1983) and occyte ovulated in sexually immature calves are capable of development to full term leading to birth of young one when transferred to sexually mature recepient (Seidel *et. al.*, 1970). However, the report on the effect of synchronising and superovulatory treatment on potential prepuberal buffalo ovaries in respect of folliculogenesis and ovulation is lacking. In this experiment response of prepubertal gonads were tested in terms of folliculogenesis and ovulation among buffalo heifers using PGF_2 alpha and PMSG combination.

MATERIALS AND METHODS

Eight murrah buffalo heifers of 12 month age were employed for this study from the animal herd of National Dairy Research Institute, Karnal, India, and were maintained under general herd managemental condition. All heifers were injected (im) 1st dose (30mg) of prostoglandin Lutalyse, Upjohn Ferrungway, crawley, sussex, U.K.) followed by 2500IU (im) of pregnant mare serum gonadotrophin (PMSG, Folligon, Intervet international BV, Boxmeere Holland) 14th day of prostaglandin administration. Second dose (25mg) of prostaglandin was injected (im) on 16th day of 1st PGF_2 alpha injection. The genitalia were examined per rectum condition and a potent vasectomized bull was paraded at 3 hour interval detection of estrus. Four heifers (BS, DS, FS and HS), Constituting experimental group were injected (iv) 200ug synthetic gonadotorophin releasing hormone (Fertagyl, intervet, Boxmeere, Holand) after 12 hours of detection of estrus. The

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remaining four heifers (AS, CS, ES and GS), Constituting control group received (iv)2ml saline solution 12 hrs. after detection of estrus.

RESULTS AND DISCUSSION

(a) Esturs behaviour and follicular response:

The injection of 1st PGF_2 alpha could not bring any change in behaviour and genital condition. The ovarian enlargement associated with follicular growth, uterine tonicity, congestion in vaginal mucus membrane, oedema and enlargement of external genitalia was observed from 24 hrs(GS), 48hrs (AS, BS, FS and HS) and 72 hrs(CS, DS and ES) of PMSG administration. The changes intensified further till the exhibition of induced estrus. GnRH administered at induced estrus in treatment group caused synchronized ovulation (Table). The effect of PMSG in terms of ovarian enlargement within 24 to 72 hrs of its administration, subsequent follicular growth, ovulation and luteal tissue formation was compararable with results obtained in cattle heifers of 11 to 12 months age receiving PMSG as superovulating compound(Takahashi 1983). The variation of superovulatory response in respect to interval from PMSG injection to ovarian changes, follicular growth, onset of estrus and duration of estrus among individual heifers may be due to the differences in the ovarian status among these heifers at the time of PMSG administration. This could be in terms of the presence of follicles of various sizes and different stages of development, as the rate of development of different sized follicles are different in response to PMSG injection (Monniaux *et. al.,* 1983).

(b) Ovulatory response of GnRH:

The nonsignificant difference in terms of total number of ovulation among both experimental and control group heifers reveals that the injection of GnRH at estrus have no influence on total number superovulation in cow (Voss *et. al.,* 1983, Parado *et. al.,* 1984) and calves (Jillela and Baker 1981) However, the difference recorded between two groups in respect of time sequence of ovulation may be due to the fact that GnRH injection at induced estrus caused synchronized ovulation by reducing periods between onset of estrus to ovulation and also from 1st to last ovulation as observed in heifers (Takahashi and Kanagawa 1983) and cows (Prado *et. al.,* 1984) and ewes(Walker *et. al.,* 1986). The prolonged follicular phase and delayed ovulation spreading over 100 hours in control group heifers is comparable to the observation recorded in buffaloes after induced estrus receiving PMSG as superovulating compound (Karaivanov, 1986).

A second wave of follicular development of 9th to 11th day of PMSG injection among all heifers of experimental while in only two heifers(ES and GS) in control group and exhibition of strong estrus in one heifers of each experimental(FS) and control(ES) group might be due to the fact that PMSG with its long life (Saumande, 1978) consistently stimulated a large number of growing follicles leading to their development and maturation on different day of PMSG administration. A similar biphasic folliculogenesis expressionn of 1st estrus on 4th to 5th day and 2nd estrus on 7th to 11th day in PMSG treated cow (Saumande and Chup in 1987) and goat(Armstrong *et. al.,* 1983) have also been reported. The observation reveals that buffalo heifers at 12 month of age have

developed intrinsic mechanisms to evoke a response of folliculogenesis and ovulation in response to tropic hormone administration.

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Table. Estrus behavior and ovarian response in superovulated buffalo heifers (Mean ±SE)

Heifers	PMSG inj. to onset of estrus (hours)	Duration of estrus (hours)	No. of Follicles Right Ovary	No. of Follicles Left Ovary	No. of Ovulation Right Ovary	No. of Ovulation Left Ovary	PMSG inj. to ovulation (hours)	GnRH/Saline inj. to ovulation (hours)
Experimental	96.00 ± 8.93	66.00 ± 6.00	3.50 ± 0.50	3.50 ± 0.50	2.75 ± 0.25	2.75 ± 0.25	106 to 144	12 to 26
Control	101.00 ± 8.06	70.00 ± 15.10	3.50 ± 0.65	4.00 ± 0.58	2.25 ± 0.21	2.50 ± 0.50	112 to 192	36 to 96

N.B: PMSG / GnRH / Saline injection to estrus, ovulation and duration of estrus is approximate.

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Morphometric Characterization of Follicles and Oocytes in Goat Ovary

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ABSTRACT

The study was conducted on 33 she goats. The size of the right and left ovaries did not show much variation. The average number of the observed follicles was 9.6. The number of oocyte recovered from right ovary was slightly more (2.73) than left (2.56). The grade IV oocytes revealed highest (14.9%) incidence. Mean diameter of the oocytes with cumulus revealed significant difference among different grades. There was a positive but non-significant correlation between size of follicle and oocytes of grade IV, III and II without cumulus but grade I oocyte revealed positive and significant correlation. The correlation of oocytes with cumulus of grade I revealed highly significant and positive correlation. The follicular diameter increased with the increase in the size of the ovary.

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Various studies on follicular oocyte aspirated from ovaries obtained from abbatoir have suggested that such oocyte retains the potential for *in-vitro* maturation. These oocytes could be used as a source of embryoes for embryo-transfer technology in livestock (Pineda and Bowen 1980). Leibfried and First (1979) reported a direct relationship between morphological characteristics of follicular oocyte and their ability to mature *in vitro.*

In view of the above this study was undertaken for morphometric characterisation of different grades of oocytes and to study the association of oocyte morphology with their respective follicular diameter.

MATERIAL AND METHODS

The reproductive organs of 33 she goats were collected from slaughter house and brought to laboratory in phosphate buffered saline (PBS) in ice box. The following measurements were taken.

1) weight of right and left ovaries,

2) length, width and thickness of ovaries (by dial calipers). The size of ovary represented the Length + Width + Length divided by 3.

3) the number of follicles in right and left ovaries, and

4) the diameter of follicles (by dial calipers).

The oocytes from each measured follicles were aspirated with the help of 5 ml glass syringe and 21 gauze needle. The oocytes in PBS were poured in a clean watch glass and examined under stereozoom microscope. These were classified into four grades as below:

Grade I Oocytes having complete, thick, more than 3 layers of compact cumulus all around the zona pellucida.

Grade II Oocytes having partially thick, 1-2 layers of cells around zona pellucida.

Grade III Oocytes with broken incomplete cumulus cells.

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Grade IV Nacked oocytes enclosed by zona pellucida only.

The oocytes were measured by occular micrometer standardized with stage micrometer.

The data were analysed statistically for mean, standard error (SE,) analysis of variance and Duncan's Multiple Range test (DMR).

The simple correlation was calculated between the diameter of the follicle, the oocyte (different grades) and the size of ovary.

RESULTS AND DISCUSSION

The perusal of data did not show much variation in mean values of the weight and size of left and right ovaries. The number of follicles observed in right and left ovaries varied from 3-18 with an average of 9.6. The follicular number observed in smaller ovary was lesser than the larger one.

The number and the percentage number of different grades of oocytes recovered from follicles of goat ovary with the mean number of oocytes recovered per ovary is presented in Table 1. The average number of oocytes recovered per ovary was 2.54. The number of oocytes recorered from right ovary was slightly greater (2.63) than the left (2.45). The number of oocytes per ovary in this study was slightly higher than reported by Chakravarty *et. al.,* (1994) and Singh (1988) as 2.08 and 2.41 oocytes per ovary, respectively.

The percentage of different grades of oocytes recovered in this study showed lower incidence (11.6%) of grade II and III but a higher (14.9%) of grade IV. The results were comparable with those recorded by Chakravarty *et. al.,* (1994), Balasubramanian *et. al.,* (1991) and Singh and Sharma (1991). In contrast, Mogas *et. al.,* (1992) found lowest incidence (11.2%) of grade I and the highest of grade II (42.9%) oocytes.

The overall mean diameter for oocyte without and with cumulus was 159.99 um and 221.05 um respectively and the over all follicular diameter 3.60 mm. There was no significant difference in mean diameter of different grades of oocyte without cumulus. However, it was significant ($P<0.05$) for oocyte with cumulus. The difference in follicular diameter having different grades of oocytes was also significant ($P<0.05$). The DMR test revealed that the mean diameter of grade III oocyte with cumulus was significantly lower ($P<0.05$) than the size of grade I and II and the diameter of grade I oocyte was significantly higher than oocyte of remaining two grades. The DMR test further showed that the follicular diameter of grade IV was significantly smaller ($P<0.05$) than the rest of 3 grades. The mean diameter of follicle of grade I,II and III decreased gradually, however, the difference was not significant (Table 2).

The average diameter of follicles and oocytes in the present study was in line with the report of Singh (1988) who reported these values to be 2.41+0.06 mm and 158.76+0.90 um respectively in goat. However, the size of oocyte of goat observed in the present study was considerably larger in diameter as compared to buffalo (77.9-95.1 um) as reported by Salvaraj *et. al.,* (1992).

While comparing the oocyte of four grades, a pattern of gradual increase in diameter of oocyte with cumulus was observed. Whereas there was no such pattern noticed in the oocyte without cumulus, the size of oocyte with cumulus differed for all the 3 grades (Table 2). Probably the size of oocyte did not change with maturation.

There was a positive but non-significant correlation (P<0.05) between follicle size and grade IV, III and II oocytes without cumulus. However, grade I oocyte revealed a positive and significant correlation. The correlation of oocyte with cumulus was also positive and non-significant for grade III and II but highly significant (P<0.01) and positive for grade I oocyte. The over all correlation for follicle diameter and oocyte without cumulus was positive and sinificant. The correlation pattern revealed that the diameter of follicle increases with maturation of oocyte. the correlation between follicle diameter and size of right ovary was positive but nonsignificant in contrast to left ovary where it was positive and significant. Thus follicle diameter increase with increase in size of ovaries. The comparable data on correlation appears to be meagre.

Table 1. Grades of oocytes recovered from ovaries of goat

Attribute	Right		Left		Total	
	Number	%	Number	%	Number	%
Ovaries Examined	33	—	33	—	66	—
Visible Follicles	314	—	298	—	612	—
Follicles punctured	170	—	164	—	334	—
Oocytes recovered						
Grade I	18	10.58	23	14.02	41	12.27
Grade II	23	13.52	15	9.15	38	11.38
Grade III	25	14.70	14	8.54	39	11.68
Grade IV	21	12.35	29	17.68	50	14.97
Total	87	51.17	81	49.39	168	50.29
Mean Number of oocytes recovered/ovary	2.63	—	2.45	—	2.54	—

Table 2. Mean and SE of different grades of oocytes with respective follicle in goat

Grade	Follicle (cm)		Follicle (cm)
	Without cumulus	With cumulus	
I	163.07^{a} + 1.47	253.16^{a} + 5.55	3.94^{a} + 0.09
II	159.88^{a} + 2.96	219.12^{b} + 5.86	3.67^{a} + 0.13
III	158.05^{a} + 4.07	190.88^{c} + 5.01	3.61^{a} + 0.24
IV	158.89^{a} + 1.09	—	3.21^{b} + 0.15
Overall Mean	159.99 + 2.39	221.05 + 5.47	3.60 + 0.15.

* Means having same superscript do not differ significantly (P<0.05)

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Preliminary Studies on Embryo transfer in local breeds of Goats of Gujarat

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ABSTRACT

Three cyclic goats were superovulated using 133 mg Folltropin-V injected I/m in 8 divided doses over a 4 days period (16.6 mg per injection). Four recipients were synchronised along with the donors using 7.5 mg prosolvin. The embryos were surgically collected from donors after 72 hrs. post breeding and were transfered into the recipients ipsilateral to the Corpus luteum (CL). The total number of anovulatory follicles, CL, fertilized ova, unfertilized ova and transferable embryos recovered from the three donors were studied. The progesterone and Estradiol levels were estimated during the pretreatment and treatment period in all the three donors and also in the recipients.

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Superovulation and embryo transfer in goats has been achieved by using FSH or PMSG by various workers (Agrawal *et. al.*, 1982, Nandi *et. al.*, 1990, Krisher *et. al.*, 1994, Sarmah *et. al.*, 1996). Folltropin-V has been used successfully and is being used extensively for superovulation in cattle and buffaloes (Madan 1990, Mishra *et. al.*, 1992, Sarvaiya *et. al.*, 1992 and Chauhan *et. al.*, 1994). However there are no reports on the use of Folltropin-V in goats. Therefore the present experiment was planned to study the effect of Folltropin-V for superovulation in goats for embryo transfer.

MATERIALS AND METHODS

Three normal cycling female goats of Surti or Marwari breed between 25-30 kg body weight were selected as donor. The superovulatory treatment was started between day 13 to 15 of the oestrous cycle and Folltropin-V (Vet Pharma Canada) was administered at the rate of 16.6 mg per injection in morning and evening for four days (133 mg total does). Prosolvin (7.5 mg) (UpJohn) was injected I/m along with 6th injection of Folltropin-V to the donors. The recipients were injected with prosolvin 24 hrs earlier than the donors. The animals were checked for oestrus using a buck. At oestrus, the donors were bred by the proven fertile bucks and 750 I.U. Chorulon (Inter care) was injected to each donors.

The donors were laparotomised by mid ventral incison and the reproductive organs were explorated. A 5.0 cm long thin sterile plastic cathetor was placed in the fimbriated end of fallopian tube. 5 ml of Ringer's solutions was infused through utero-tubal junction towards fimbriated end of the fallopian tube and the fluid was recovered in small sterile petridishes (Agrawal, *et. al.*, 1982). The number of Corpus luteum (CL) and unovulated follicles were counted on both the ovaries. The embryos were located under stereomicroscope and the detailed morphology was studied under inverted phase contrast microscope. The recipients were operated in the same manner as donors. The reproductive organs were located and embryos were transfered either through fimbriated end or through uterotubal junction with the help of a microsyringe,

ipsilateral to the CL. For endocrinological studies, the blood samples were drawn by puncturing jugular vein. The serum was harvested by centrifugation and preserved at - 20° C till the hormone assay. The radio immuno assay (RIA) of progesterone (P) was performed by coat - a - count method and estradial (E) levels were assayed using double antibody technique using DPC (Diagnostic Product Corporation, U.S.A.) RIA kits.

RESULTS AND DISCUSSION

Superovulatory response in donors and embryo transfer in recipients :

All the three donors exhibited super ovulatory oestrus within 24 hrs after the last injection of Folltropin-V. The total number of anovulatory follicles, CL, fertilized ova, unfertilized ova and transferable embryos recovered from the three donors were 19,53,25,29 and 25 respectively. The transferable embryos were between 8-16 cell stage. In the present study, the number of transferable embryos recovered were higher than those reported by Sarmah *et. al.,* (1996), Krisher *et. al.,* (1994) and Nandi *et. al.,* (1990).

Embryos were surgically transfered through fallapian tube in two recipients and through uterotubal junction in the remaining two recipients. Number of embryos transfered varied from 1-4. Two recipient goats became pregnant and delivered one male and one female kids.The 50% conception rate after surgical embryo transfer is slightly lower than that reported by Agrawal *et. al.,* (1982).

In the donor goats the progesterone levels remained high during the pretreatment (6-12.5 ng/ml) and treatment period (5.4 to 15 ng/ml) and dropped to a very low level on the day of oestrus (0.38-0.82 ng/ml). The level again increased on the day of embryo collection (19.0 to 22.5 ng/ml). The levels of estradiol increased from the pretreatment level (5.5 to 14.8 pg/ml) with the advancement of treatment and reached to a maximum level (35.2 to 80.0 pg/ml) as the day of super oestrus and later declined on the day of embryo collection (10.8 to 31.2 pg/ml). Individual variation were observed in hormone levels, however, the pattern was similar in all the three donors. The levels of P were higher in the present study than those reported by Akinlosotu and Wilder (1993). However, the pattern of progesterone secretion was similar. Borque *et. al.,* (1993) studied the progesterone level upto day 5 after oestrus in superovulated goats and reported increasing level of progesterone.

In recipient goats it was observed that during oestrus the estradiol levels were high (15.0 to 24.0 pg/ml) in all the recipients where as the P levels remained significantly low (0.14 to 0.6 ng/ml). However, from the day of embryo transfer onward, till day 55, the progesterone levels remained high in two recipients who ultimately turned out to the pregnant. In the other two non-pregnant animals the progesterone levels were found to be low or irregular after day 20 post oestrus. The progesterone levels reported in the present study in pregnant animals after day 20 are in close agreement with the earlier findings of Deshpande and Mehta (1992).

It was concluded that Folltropin-V can be successfully used for superovulation and embryo transfer in goats. The P levels could be of great value for monitoring pregnancy in recipient goats.

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Studies on circulating levels of Thyroid Hormones in periparturient female Camel (Camelus dromedarius)*

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ABSTRACT

Circulating levels of Thyroxine (T4) and Triiodothyronine (T3) were estimated from serum samples of eight pregnant camels during one month before and two months after parturition. The hormones were analysed by radio-immunoassay technique. The average level of T4 was a little higher during first fortnight of prepartum month, otherwise the T4 and T3 levels persisted at a constant level during pre and post partum period (Range T4-64.38±6.58 to 80.86±8.10 ng/ml and T3-12.0±0.26 to 16.6±0.41 ng/ml). Stage to stage variations were not statistically significant.

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Thyroxine (T4) and Triiodothyronine (T3) are the main biologically active hormones secreted from thyroid gland under the influence of thyroid stimulating hormone (TSH) from anterior pituitary. The role of these hormones in control of metabolic rate is well documented. A deviation of these hormones from basal levels may lead to serious complications. Physiological levels of thyroid hormones in female camel have been reported during follicular cycle (Agarwal *et. al.,* 1995) and pregnancy (Elias *et. al.,* 1984 a,b; Agarwal *et. al.,* 1989) but information on thyroid hormones around parturition is lacking. Therefore, this study reports the levels of camel thyroid hormones during pre and post partum periods.

MATERIALS AND METHODS

Eight single humped she camels (Camelus dromedarius) in the last month of gestation were selected for this study. Blood samples were collected on days 28, 21 14, 7, 5, 3, 1 prepartum and 1, 3, 6, 13, 20, 27, 34, 41, 48, 55 and 62 postpartum. Serum was separated and stored at -20°C till analysed. For the assay of T4 and T3 specific RIA kits were procured from Bhaba Atomic Research Centre (BARC), Bombay. All the reagents supplied in the kits were freshly reconstituted on the day of estimation. The proceduaral steps were followed as per the protocol supplied with the kit with minor modifications. The sensitivity of the assay for T4 and T3 was 31.0 pg and 3.1pg, respectively. The intra-assay coefficient of variation was 4.20% for T4 and 5.40% for T3, whereas inter-assay variation (C.V) for T4 and T3 was 12.30 and 10.30 per cent, respectively. The results were analysed statistically to know the variation between the stages using completely randomised design and students "t" test in ECIL Micro 32 Computer.

RESULTS AND DISCUSSION

The thyroxine (T4) levels were slightly higher (around 80 ng/ml) during the first

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fortnight of last month of gestation which later declined to 65-70 ng/ml during the week preceding parturition. During post partum period, the levels did not exhibit any particular trend and the concentration fluctuated between 64 and 74 ng/ml (Fig.1). The stage to stage variation were not significant statistically.

The triiodothyronine (T3) levels showed minor fluctuations from 1.93±0.18 to 2.51±0.39 ng/ml during prepartum and from 1.50±0.33 to 2.53±0.28 ng/ml during postpartum stages. The levels were almost constant throughout pre and post partum stages except for minor decline around parturition and at two months post partum (Fig.2). The stage to stage differences were not significant statistically. Similar to these results, mild elevation of thyroid hormones during terminal month of pregnancy in camel has been reported (Agarwal *et. al.*, 1989)

The exact role of thyroid hormones in the process of parturition is not established. It has been shown that the increased thyroid activity may utilize the nitrogen metabolic pool of an available source of energy, thus meeting the requirements of the tissues of the reproductive system and of the growing embryo (Hafez, 1968). The present results indicated that the thyroid hormones were not influenced by the act of parturition but might play some role on a permissive rate on tissues for the action of other hormones directly involved in the process of parturition.

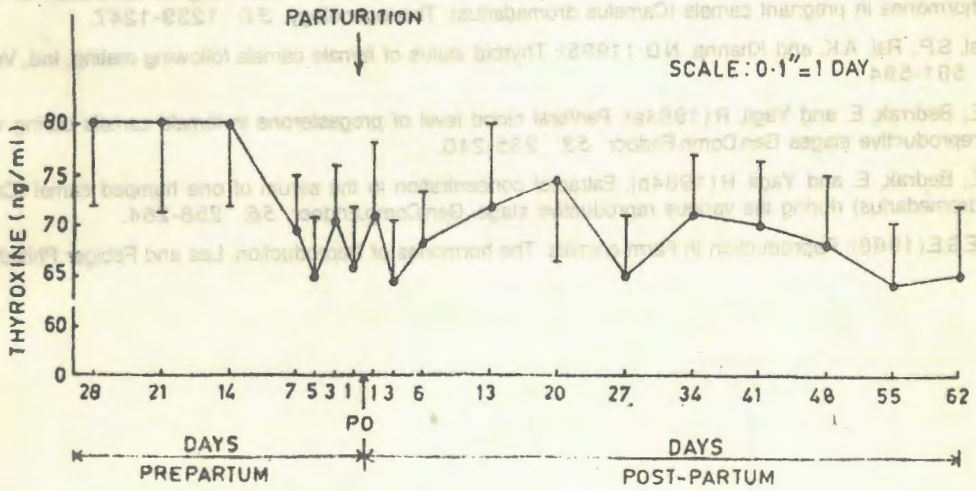

Fig. 1. Mean (±SEM) Thyroxine (T_4) in Periparturient Camels*

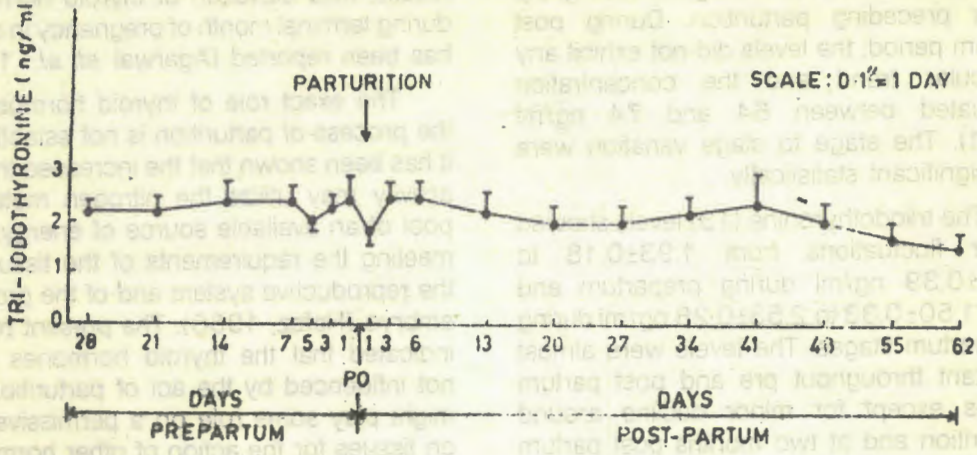

Fig. 2. Mean (±SEM) Tri-Iodothyronine (T_3) in Periparturient Camels

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Success of Treatment and Dam survival in Bovines with Precervical Uterine Torsion.

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ABSTRACT

A total of 34 animals (buffaloes = 20 and cows = 14) suffering from pre-cervical uterine torsion were evaluated retrospectively. The animals were categorised into three groups depending upon the onset of labour pains. In group-I (upto 36 hrs), detorsion with plank method was achieved in 81.8 per cent cases, out of which only 66.6 per cent could be delivered per-vaginum. Although, detorsion was achieved in all the cases in group-II (36-72 hrs), yet, caesarean section had to be performed in all as the cervix failed to dilate. In group-III (beyond 72 hrs), detorsion was achieved in 66.6 per cent cases but without any per-vaginal delivery. The overall dam survival rate in the three respective groups was 54.5, 50.00 and 47.3 per cent. It was concluded that the pre-cervical uterine torsion had a detrimental effect on the cervical dilatation and majority of cases required surgical intervention for delivery of the fetus.

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Torsion of uterus is a frequently occuring complication of advanced pregnancy in cattle and buffaloes (Prabhakar *et. al.,* 1994). The position of torsion could either be pre-cervical or post-cervical with majority of buffaloes having post-cervical uterine torsion (Singh, 1991). Success of treatment in terms of per-vaginal delivery and dam survival varied with the duration of the problem (Pearson, 1971). Survival rate of the dam with post-cervical uterine torsion has been found to decline linearly with the increase in duration of labour pains (Dhaliwal *et. al.,* 1991). No information, however, is available about the effect of site (pre or post-cervical) of uterine torsion on the success of treatment.

The present study was, therefore, conducted to record the success rate of treatment and dam survival in cows and buffaloes with pre-cervical uterine torison.

MATERIALS AND METHODS

A total of 34 animals having pre-cervical uterine torsion (Buffaloes = 20 and Cattle = 14) from amongst those presented for the treatment of uterine torsion (n = 520) during the period 1985-1991 were evaluated retrospectively. The cases with duration of dystocia for less that 36 hrs, 36-72 hrs and more than 72 hrs were placed into groups I, II and III respectively. On presentation, the cases were clinically evaluated and those without any utero-omental adhesions were subjected to detorsion by Sharma's modified method (Singh and Nanda, 1996). while the others with utero-omental adhesions and considered unfit for rolling were subjected to ceasarean section (C.S.). C.S. was also performed in some of the other cases where either detorsion failed to occur or cervix failed to dilate following detorsion. In addition, the letdown of milk and relaxation of pelvic ligaments was also observed to find its relation with the success of treatment. Dam survival rate following treatment and fetal delivery per-vaginum or through C.S. was recorded. Since, majority of cases were handled at field level prior to presentation,

survival of the dam was considered to be the criteria for success of treatment.

RESULTS AND DISCUSION

of 520 cases of uterine torsion recorded during the period, 34 (6.5%) were of pre-cervical uterine torsion. Singh (1991) and Prabhakar *et. al.,* (1994) have reported lower incidence of pre-cervical as compared to post-cervical uterine torsion in buffaloes while Pearson (1971) has also reported a lower incidence of pre-cervical uterine torsion in cattle. The attachment of broad ligaments to the genitalia is limited from ovaries upto only anterior half of the cervix, which might keep the pregnant genitalia balanced (Singh, 1991). Lack of this type of support posterior to the cervix might leave the gravid uterus imbalanced and more prone to rotation, thus, the higher incidence of post-cervical uterine torsion in buffaloes.

Enlargement of the udder and relaxation of pelvic ligments were recorded in relation to the duration of labour pains in animals with pre-cervical uterine torsion. It was evident that in fresh cases (group-I), 90 per cent had complete letdown of milk and had relaxed pelvic ligaments. In delayed cases (group-III), the milk in udder has been resorbed and pelvic ligaments again tightened in 84.2 per cent animals, while in group-II, the udder was partially shrunken and pelvic ligaments were partially retightened in all the animals. Similar findings were also observed in buffaloes having post-cervical uterine torsion (Prabhakar *et. al.,* 1995).

In group-I buffaloes (less than 36 hrs old cases), irrespective of the degree of uterine torsion, detorsion with plank method (Singh and Nanda, 1996) was achieved in 81.8 per cent cases. However, only one animal (11.1%) had completely dilated cervix at detorsion. The cervix in five (55.5%) more animals dilated within 24 hrs following medicinal treatment . Per-vaginal delivery of dead fetuses was affected in these six animals. Cervix failed to dilate in the remaining three cases, which were then subjected to C.S. Contrarily, successful detorsion (96.7%) and per-vaginal delivery (89.4%) could be achieved following detorison with plank method in bovines with post-cervical uterine torsion of less than 36 hrs duration (Dhaliwal *et. al.,* 1991).

In group-II although, detorsion was achieved in all the attempted cases, yet, cervix did not dilate in any wherein C.S. had to be performed. Likewise, in sufficiently delayed cases (group-III), detorsion was achieved in 66.6 per cent cases, but per-vaginal delivery was achieved in none. All the animals where C.S. was performed either following unsuccessful rolling or where cervix did not dilate after detorison, failed to survive. However, 81.8 per cent animals, where C.S. was performed without attempted detorsion, could survive. Stress of ill-planned rolling before surgery (Nanda *et. al.,* 1991) or toxaemia due to tissue degeneration may lead to decline in the survival rate. Prolongation of problem has been found to reduce the success of detorsion and per-vaginal delivery of fetus in torsion affected buffaloes (Dhaliwal *et. al.,* 1991; Prabhakar *et. al.,* 1995). Significantly lower rate of per-vaginal delivery in animals affected with pre-cervical uterine torsion suggested its detrimental effect on cervix in terms of its dilatation, which occurred perhaps due to rapid setting of degenerative changes owing to severe occlusion of blood vessels in broad ligaments in pre-cervical uterine torsion.

The overall survival rate in the present study was 54.5 50.0 and 47.3 per cent in groups I, II and III, respectively which was much lower as compared to that

observed in animals with post-cervical uterine torsion in buffaloes (Dhaliwal *et. al.*, 1991).

It thus, appears from the above findings that the cervix fails to dilate sufficiently after detorsion of uterus in animals affected with pre-cervical uterine torsion. If the condition has existed for more than 36 hrs, C.S. should be performed without attempting detorsion to improve the dam survival.

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Clinical Study on Prolapse of Genitalia in Murrah Buffaloes

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ABSTRACT

Total 63 Murrah buffaloes with prolapsing genitalia were included in the study. Pluriparious (98.40%) and stall-fed (82.54%) buffaloes were observed to be more prone to genital prolapse than heifers and semi-stall fed or free grazing animals. Dystokia and retained placenta were found to be the two exciting causes for subsequent postpartum vaginal and uterine prolapse. Calcium and phosphorus therapy was helpful in curing the malady especially in mild cases. The modified Buhner's technique with posterior epidural anaesthesia was found to be very effective with least side effects for retaining the prolapsed mass. The buffaloes with uterine prolapse took significantly longer duration for uterine involution than the buffaloes calving normally. The exfoliative vaginal cytology showed increased number of pus and epithelial cells in all buffaloes with maximum number in severe degree followed by moderate and mild degree of cervico-vaginal prolapse.

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Prolapse of genitalia is considered as one of the major reproductive problem occurring in buffaloes causing great economic loss to the farmers. The affected animal not only produce less milk but also lose its future reproductive efficiency. The neglect in the treatment of the condition may result in permanent damage to the reproductive organs followed by their dysfunction and occasionlly death of the animal. The present study envisages clinical findings in prolapsing genitalia of Murrah buffaloes with a suitable line of treatment.

MATERIAL AND METHODS

Total 63 cases of Murrah buffaloes with prolapsing genitalia (7 nonpregnant with vaginal, 33 prepartum vaginal, 14 postpartum vaginal and 9 uterine) were studied. The prolapsed mass was cleaned, reduced and replaced aseptically. A line of non surgical treatment was designed based on the degree of the prolapse. The vaginal and uterine tears were sutured by chromic catgut No. 1. Modified purse string suture were placed by Buhner's needle over vulva for retention of the reposed organ for 3 to 8 days, where necessary. Involution of uterus was studied in 8 buffaloes with uterine prolapse and 6 buffaloes with normal parturition. The exfoliative vaginal cells were examined before starting the treatment, and on 2nd, 4th and 6th day post treatment of 21 animals suffering from varied degree of cervico-vaginal prolapse. The prolapsed mass was thoroughly cleaned and then douched with 50 ml normal saline. The douchings were centrifuged and smear was prepared with the sediment and stained with the Leishman stain to study the exfoliated vaginal cells.

RESULT AND DISCUSSION

Clinical Findings

The present study revealed that the 98.40 per cent of the prolapsing buffaloes were pluriparous. Excessive stretching of the pelvic ligaments and the loosening of the vaginal muscles in the earlier calvings may be the probable reason fo the higher

incidence of this malady in the pluriparous animals. Stall fed animals were observed to be suffering more (82.54%) from the genital prolapse as compared to the semi-stall fed. Lack of exercise and close confinement to the stanchion were reported to be the predisposing factors for genital prolapse in cattle also (Holland and Knox, 1967). Reduced appetite was observed more in severe degree of the prolapse cases. Recurrence of the prolapse of vagina through previous gestation was 33.33 per cent. Kelkar (1984) also reported 47.05% recurrence of vaginal prolapse in prepartum buffaloes. Dystokia and retained placenta were the two exciting causes for the genesis of postpartum prolapse of genitalia, as 35.68% vaginal and 33.33% uterine prolapses were prelude to the same. Total 42.86% of the prolapse cases were already handled unsuccessfully, and in all such cases inflammation and injury to prolapsed mass was noticed. Most of the prolapsing animals were with normal body condition (84.13%) and some were even obese (14.29%). It is against the belief that weak and debilitated animals are more prone to prolapse of genitalia. Mishra (1976) and Pandit *et. al.*, (1982) also made similar observations.

The severe degree was most common in uterine (77.78%) than the vaginal (18.52%) prolapse. Bladder distension with retention of urine was 55.56% in animals showing uterine prolapse, whereas it was only 15.15% in prepartum vaginal prolapse. The maximum blader involvement in uterine prolapse cases may be due to the degree of protrusion of genitalia which caused extensive pressure on urethra in these animals. Retention of urine due to prolapse of genitalia has also been reported in cattle (Deka and Sharma, 1977)). The rectal prolapse was also associated with vagina prolapse in nonpregnant buffaloes (42.86%), followed by pregnant (18.18%) and postpartum (14.29%). This could be due to more number of the chronic vaginal prolapses in the nonpregnant buffaloes as the acute genital prolapses were not associated with the rectal prolapse. Pandit (1979) attributed associated rectal prolapse to the chronic and recurrent straining due to vaginal prolapse. Inflammation, oedematous swelling and injury of the prolapsed mass was noticed in majority of the moderate and severe degree cases. Purulent discharge was observed in 59.26% animals with vaginal prolapse. It was difficult to ascertain as to whether infection resulted in prolapse or prolapse occured subsequent to infection. Sharma *et. al.*, (1977) reported vaginal prolapse in a buffalo as a result of urinary tract infection.

Treatment

The 95.24% prolapse cases were cured successfully between 3 to 16 days of treatment. Of the 17 buffaloes with severe degree of prolapse, 3 died due to severe injury and haemorrhage. Calcium and phosphorus therapy was helpful in curing the malady especially in mild cases. The modified Buhner's technique with posterior epidural anaesthesia was found to be very effective with least side effects for retaining the prolapsed mass. In moderate and severe degree of genital prolapse, removal of irritant purulent material and local application of Wokadin/Enrocin solution facilitated early recovery. In severe cases of genital prolapse the retention sutures together with supportive therapy was of better therapeutic value. Progesterone therapy was helpful as a preventive or therapeutic agent in cases of prepartum vaginal prolapse. For contraction of the uterine wall after proper reposition of prolapsed uterus, oxytocin injection showed good results. The buffaloes with uterine prolapse took significantly

longer duration for uterine involution than the buffaloes calving normally.

During the prolapse, large number of pus cells were found in all degrees. The maximum number was observed in severe degree followed by moderate and mild degrees of the prolapse. As the inflammatory condition or injury reduced, the number of pus cells and epithelial cells also reduced correspondingly. The RBCs gradually decreased as the condition improved. Similar observations were also made in inflammatory condition of vagina by Subramanian and Pattabiraman (1988). In normal parturient buffaloes the duration for complete uterine involution was 24.16±0.84 days in comparison to 32.50±0.64 days in buffalaoes with uterine prolapse. These findings are in resemblance to the findings of Paul (1995) who also reported less duration for complete uterine involution in normally parturient Murrah buffaloes as compared to abnormal calvings.

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Studies On the Efficacy of "Deltamin" Feed Supplement On Fertility in Cross-bred Cows.

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ABSTRACT

"Deltamin" therapy was tried in recently calved Crossbed Cows and late pubertal crossbred heifers. 83.33 per cent of the recently calved cows came in heat within an average period of 69.66 days post-treatment, whereas 83.33 per cent of the post-partum anoestrous cows came in heat within an average period of 70.40 days and late pubertal heifers came in heat within an average period of 13.2 days post-treatment. The conception rate was 80 per cent, 100 per cent and 100 per cent in recently calved cows, post-partum anoestrous cows and late pubertal heifers respectively. The inseminations required per conception were 1.25, 1.4 and 1.2 in recently calved cows, post-partum anoestrous cows and late pubertal heifers, respectively.

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Malnutrition increases the time taken to attain optimum body weight and thereby influence the age of puberty. Likewise, the resumption of post-partum reproductive cycles in lactating cows is dependent upon the average negative energy balance during the first three weeks of lactation. Greater the average negative energy balance longer is the interval from calving to first ovulation (Thatcher 1986).

"Deltamin" feed supplement contain amino acids, Vitamins, major and trace minerals, which may regulate the average negative energy balance. Therefore, the efforts have been made to findout the efficacy of "Deltamin" in cross-bed cows.

MATERIAL AND METHODS

The experiment was conducted on 36 crossbred females belonging to Cattle Breeding Farm, Borgaon Manju, P.K.V., Akola. After thorough gynaecoclinical examination the experimental animals were divided into three groups, as detailed below.

Group I : Recently calved cows: 12 recently calved with healthy genital organs.

Group II : Post-Partum anoestrous cows: 12 cows which failed to exhibit oestrus 60 days or more post-partum and showed smooth inactive ovaries.

Group III : Late Pubertal heifers: 12 pubertal heifers above 2 years of age, with body weight 200 kg and above but failed to exhibit oestrous and showed smooth, inactive ovaries and atonic uterus.

All the three groups were further subdivided into two sub groups, each consisting of 6 animals

a) Experimental group received 'Deltamin' treatment

b) Control group received no treatment.

Feeding of Deltamin

Deltamin feed supplement (Contanental pharmaceuticals, Calcutta) was administered

as an electuary with jaggary 40 g per animal daily for 21 days. In group I cow it was administered from day 5th post-partum onwards for 21 days, whereas in group II and III it was administered for 21 days commencing day 1 of treatment. Animals were watched for heat daily from the commencement of treatment.

RESULTS AND DISCUSSION

Out of the six cows treated with Deltamin in group I five responded to treatment. They came in heat within an average period of 69.66 days. One cow did not respond to treatment. Thus oestrus was induced by Deltamin therapy in five out of 6 cows, with a response of 83.33 percent.

Out of the five cows those came in heat and were inseminated four cows conceived with a conception rate of 80 percent, requiring 1.25 inseminations per conception. In control group out of 6 cows only one came in heat and conceived.

In group II, out of the 6 cows treated with Deltamin five (83.33 percent) came in heat within an average period of 70.40 days. One cow did not respond to treatment.

All the five cows those came in heat were inseminated and conceived with 100 per cent conception rate, requiring 1.4 inseminations per conception whereas in the control group only two cows came in heat within an average period of 69.5 days and conceived.

In group III out of the 6 late pubertal heifers treated with Deltamin five (83.33 percent) responded to treatement. They came in heat within an average period of 13.2 days. One heifers did not responded to the treatment. All the five heifers those came in heat conceived with a conception rate of 100 per cent requiring 1.2 inseminations per conception.

In the control group only two heifers exhibited oestrous within an average period of 14.5 days but non of them conceived during this period.

Trace elements like copper, cobalt, iodine etc. has direct effect on the reproduction. Sane *et. al.*,(1958) reported infertility due to copper deficiency in Gir heifers, whereas Laing (1970) recorded irregular osestrous periods and completely quiscent ovaries in hypocuprosis cows. Wagner (1962) reported a case of cobalt deficient dairy herd with a higher incidence of silent heat.

The forgoing literature indicates the importance of amino acids and trace elements in normal reproduction. The feed supplement "Deltamin" contains some essential amino acids and trace elements not only that, it takes care of the vitamin A and vitamin E deficiency, which affects the reproduction.

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Factors affecting some of the reproductive traits in German Angora Rabbits

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ABSTRACT

The study is based on the data collected from Govt. Angora Rabbit Breeding Farm, Kandbari in Himachal Pradesh. Reproductive traits viz., age at first kindling, gestation period and litter-size at birth were taken into consideration for the effect of year of birth, season of birth and age at first shearing. The year of birth affected litter size at birth whereas season affected litter size at birth and age at first kindling. The regression of age at first shearing did not affect these traits at all. However, these factors did not influence gestation period at all.

Angora is the one of the oldest breeds of domestic rabbits and has infact kept for its fine wool production for many hundred years. The angora character is supposed to be due recessive gene expression. Fecundity is other main character for which rabbits are known to be. However, it is lower in Angora rabbits as compare to meat type. In the present study, attempts have been made to study the effect of year of birth, season of birth on the reproductive traits. Viz., age at first kindling, gestation period and litter-size at birth.

MATERIAL and METHODS

The date on 345 angora rabbits maintained at Govt. Angora Rabbit Breeding Farm, Kandbari (H.P) from the period (1986-90) were collected. The years were further sub-divided into four seasons viz., winter, summer, rainy and autumn. The effect of partial regression on age at first kindling and gestation was also calculated. The data were analysed through least-squares analysis of variance (Harvey 1990) to see effect of season and year on these reproductive traits using the following model:-

$ijk = u + Pi + Sj + Aijk + eijk.$

Yijk = K_{th} individual born in jth season and i_{th} period.

u = Overall mean

P_i = Effect of i_{th} period or year

S_j = Effect of j_{th} season.

A_{ijk} = Partial regression of age at first shearing.

RESULTS AND DISCUSSIONS

The results are presented in the table. The age at first kindling averaged 254.96±3.90 days with 21.8% of C.V. The years do not have any effect on age at first kindling. However, season had significant effect ($P< 0.05$) on the trait. It was recorded highest in winter and lowest in autumn. These changes may be due to artifically controlled matings in different seasons and managemental factors. Age at first shearing did not have effect on this trait.

The gestation period averaged 30.60±0.08 days with 1.7% of C.V. It

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indicates that the traits had very little variations. The results fall within ranges reported by Gupta (1987) and Khali and Mansour (1987). However, Gaur (1989) reported it towards little higher side than the present findings. The year of birth, season of birth and regression of age at first shearing did not have any significant effect on it. It seems that gestation length is the stead-fast trait and which is not likely to be affected by genetic and not-genetic factors.

The overall mean for litter-size at birth was recorded at 4.85±0.16 with 33.2% of coefficent of variation. The present estimate was in close agreement with the findings of Garcia and Magofke (1986) Singh (1986) Afifi and Emara (1987) and Gaur (1989). Year of birth had significant effect on this trait. It was recorded highest in year 1986 and lowest in the year 1980. Similar findings are reported by Khalil *et al.*, (1987) and Rathore (1990). Differences in years may be due to managemental and feeding practices at different years which might have affected number of ova shedded during ovulation period.

Seasons were also the source of variations for littersize at birth. The highest was recorded in summer season whereas it was recorded lowest in autumn. The similar findings were recorded by Pinka Vova (1983) and Gaur (1989). The variations due to season in litter-size may be due to different climatic conditions in the year.

Table Least squares means for some reproductive traits in Angora rabbits.

Source of variation	Age at first kindling (days)	Gestation period (days)	Litter size at birth
Over all mean	254.96±3.90	30.60±0.08	4.85±0.16
Effects:			
1986	285.76±11.27^{b}	30.61±0.08	5.60±0.19^{c}
1987	247.13±7.37^{a}	30.58±0.08	4.46±0.24^{b}
1988	253.04±8.11^{a}	30.73±0.08	5.19±0.21^{ab}
1989	257.56±6.27^{ab}	30.65±0.05	4.95±0.14^{a}
1990	252.25±21.34^{a}	30.62±0.018	4.53±0.62^{a}
Seasons:			
Winter	253.91±8.84^{b}	30.73±0.07	5.14±0.19^{b}
Summer	250.69±5.92^{a}	30.71±0.06	5.51±0.22^{b}
Rainy	270.97±6.50^{b}	30.52±0.05	4.82±0.21^{a}
Autumn	206.33±12.25^{a}	30.91±0.08	4.32±0.38^{a}

Means with same super script do not differ significantly ($P<0.05$).

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Influence of Programmable Freezing on Cryosurvival of Awassi Ram Spermatozoa

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ABSTRACT

Comprehensive and precise estimates of sperm motion during cryopreservation are useful indicators of the viability of spermatoza. The objective of this study was to evaluate sperm kinematics of Awassi ram spermatozoa frozen under control freezing conditions by computer-assisted semen analysis technique. Ejaculates of good quality semen obtained from adult rams were pooled, extended @ 1000 million spermatozoa per ml and filled in 0.25ml straws. Samples were equilibrated for 2h at 5°C, frozen at a linear rate of -25°C per minute in a programmable cell freezer and stored at -196°C. Thawing was done at 50°C for 10 seconds in a water bath. The mean post-thaw recovery of motile spermatozoa was> 70% in two replicates. Among the replicates the effect of thawing was significant on average path velocity, straight line velocity, % medium, %linearity and %straightness but not significant on % motility, % rapid, %slow, curvilinear velocity, amplitude of lateral head displacement and beat frequency of spermatoza.

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Cryopreservation is known to adversely affect the survival of ram spermatozoa (Pontbriand *et. al.,* 1989 Joshi and Mathur, 1994; Mathur and Joshi, 1994; Salamon and Maxwell, 1995). Freezing of Awassi ram semen in straws by conventional vapour freezing and subjective assessment resulted in 42.59% post-thaw recovery of motile spermatozoa (Bhosrekar *et. al.,* 1994). An attempt has been made in this study to cryopreserve Awassi ram semen in straws under controlled conditions by programmable freezing and evaluate the post-thaw attributes by computer-assisted semen analysis (CASA) technique.

MATERIAL AND METHODS

Semen collection, evaluation and extension:

The adult Awassi rams maintained under semi-intensive management system at the Institute farm were used as semen donors in this study. Semen was obtained by the use of artificial vagina and was evaluated subjectively for semen volume, consistency, wave motion (0-5 scale), sperm concentration, motility (0-100%) and intensity of movement of motile spermatoza (0-4 scale). Ejaculates having thick consistency, rapid wave motion (+5), spermatozoa concentration>3000 million per ml, 90% initial motility with +4 rating of intensity of movement were pooled and diluted @ 1000 million spermatoza per ml (Mathur and Joshi, 1996) using egg yolk tris glycerol extender (Schmehl *et. al.,* 1986).

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Semen processing, freezing and thawing:

The extended semen samples were filled in 0.25 ml plastic straws and equilibrated for 2 h at 5°C. Equilibrated straws were frozen at a linear rate of -25°C per minute upto -125°C in a programmable cell freezer (R-204, Planer Products Ltd., U.K.) and stored in liquid nitrogen until required (Mathur and Joshi (1996). Thawing was done at 50°C for 10 seconds in a thermostatically controlled water bath (Joshi and Mathur, 1996).

Analysis of sperm kinematics:

The computerized semen analyser (HTM-S version 7.2 Y, Hamilton-Thorn Research Inc., U.S.A) was used for objective assessment of sperm kinematics of Awassi ram spermatozoa during cryopreservation. Prior to analysis the analyser was set-up by the procedure as described by Joshi and Mathur, 1996. The parameters included for CASA were %motility, %rapid (APV>25 μm/sec), %medium (10<APV<25 μm/sec), %slow (0>APV>10 μm/sec), curvilinear velocity (CLV, °μm/sec), average path velocity (APV, °μm/sec), straight line velocity (ALH, °μm/sec), %linearity, %straightness, amplitude of lateral head displacement (ALH, °μm) and beat frequeny (BF, Hz) of spermatozoa for 40 post-thaw observations in each replicate. Statistical analysis of data was done by students 't' test of two independent means after are sin transformation of values recorded in percentage (Ipsen and Fiegl, 1970).

RESULTS AND DISCUSSION

In conventional freezing the main disadvantage is that apart from setting the intial conditions of vapour temperature and liquid nitrogen level in the freezing chamber there is no further control over the process (Parkinson and Whitfield, 1987). The use of programmable freezing technique in this study overcomes this disadvantage. CASA technique provides unbiased objective assessment of sperm kinematics during the freeze-thaw process (Budworth *et al.*, 1988).

The values recorded for motility, rapid, medium and slow moving spermatozoa after dilution were 90.5 vs 90% 85.5 vs 87.5%, 2 vs 2% and 0.5 vs 0% in both the replicates, respectively. The mean post-thaw recovery of motile spermatoza was more than 70% in both the replicates. The respective drop of percent post-thaw motility in the two replicates were 17.3 vs 18.2% as compared to the values recorded at dilution stage. Although after thawing the majority of motile spermatoza were rapidly moving but the distribution between medium category was also prominent with few slow moving spermatozoa. The percent of medium category differed among replicates ($P<0.05$) but there was no significant change on percent post-thaw motility, rapid and slow moving spermatoza.

Alongwith motility, sperm velocity is the important parameters of post-thaw attributes as both are related with fertility (Aitken, 1990). The CLV, APV and SLV of diluted samples in both the replicates were 138.5 vs 144.5, 99.5 vs 101.5 and 71.5 vs 70.5 °mm/sec respectively. After freeze-thaw process the decrease of 30 vs 33.6% in CLV, 29.5 vs 35.2% in APV and 23.2 vs 29.2% in SLV was observed as compared to dilution in both the replicates, respectively. Among the two replicates the values recorded of APV and SLV after thawing were also significantly different ($P<0.05$).

The ALH was also highest at dilution (8.7 vs 8.3 °mm) but decreased by 23 vs 21.6%, respectively after post-thaw in both the replicates. It has been reported

that the rapidly moving spermatozoa with a high CLV or SLV have greater ALH (Budworth *et. al.*, 1988). The lower value of ALH recorded at post-thaw stage in this study may be attributed to the decrease in sperm velocities after the freeze-thaw process. Although the present linearity and percent straightness differed after post-thaw among the replicates ($P<0.01$) but there was no significant effect on ALH and BF of spermatozoa.

Cryopreservation of spermatozoa under controlled freezing conditions significantly improve sperm survival after thawing (Parkinson and Whitfield, 1987; Mathur and Joshi, 1996). The results obtained in this study indicate that 70% post-thaw survival of Awassi ram spermatoza could be achieved by programmable freezing and computerized assessement.

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Preservability of buffalo semen in dilutors without yolk: Effect of glycerol and sugars.

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ABSTRACT

Motility of Murrah buffalo spermatozoa in milk, tris and sodium citrate dilutors containing 3 levels of glycerol and 3 levels of 6 sugars in absence of yolk was assessed upto 72 hr. Milk and tris maintained good motility (30-40%) upto 48 h with fructose, sucrose and raffinose in presence of 3 or 6% glycerol. Higher glycerol (9%) was detrimental in milk and tris but in citrate it maintained satisfactory motility in presence of fructose and raffinose. Sugars behaved independently in different buffers. Satisfactory motility during refrigeration storage in milk and tris dilutors even in the absence of yolk was possible.

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Glycerol and sugars impart beneficial effect in presence of yolk in the dilutor during freezing (Bhandari *et. al.*,; 1983). Little information is available when sugar and glycerol are used as only protective agents in the media without yolk (Kumar 1989). An interaction between additives and extender formulation and cryoprotective role of sugar and level of glycerol in the dilutor exist (Salamon *et. al.*, 1973). A study was undertaken to record the protection afforded by 3 levels of glycerol (3, 6 and 9%) and 3 levels (1.0, 1.5 and 2.0%) of 6 sugars (glucose, fructose, xylose, raffinose, sucrose and common table sugar; containing 99.8% sucrose) in 3 extenders (milk, tris and sodium citrate) during refrigeration preservation of buffalo spermatozoa in absence of yolk.

MATERIALS AND METHODS

Semen samples were collected from Murrah buffalo bulls (age 3½-4½ yrs) maintained under uniform feedings and managemental regime at Germ Plasm Centre of the Institute. Split ejaculates (initial motility 70% and above) were extended in freshly prepared dilutor combinations (containing either of 3, 6 and 9% glycerol and 1.0, 1.5 2.0% of either of the 6 sugars i.e. glucose, xylose, raffinose , fructose, sucrose and common table sugar in milk, tris and 2.9% sodium citrate solution. Fresh cow milk was used as extender after removing the fat (Kumar 1989). Tris buffer was prepared according to Davis *et. al.*, (1963). Each dilutor combination was tested against 6 ejuculates and motility was asssessed at 24, 48 and 72 h. The means and standard errors were calculated.

RESULTS AND DISCUSSION

Refrigeration preservation was possible without yolk, in presence of glycerol and sugars, although satisfactory motility (above 40%) was maintained for a short duration. Variation in the motility and duration of maintenance of satisfactory motility was found due to buffers, possibly because of their nature and chemical composition. Good

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motility scores upto 48 hrs was maintained in milk and tris but not in citrate even upto 24 h. Only raffinose and fructose in citrate (with 9% glycerol) could maintain 30-40% motility upto 24 h. The deleterious effect of glycerol and no beneficial effect of sugars in citrate during storage was observed. Since dilutors did not contain yolk, the deterimental effect of glycerol was evident sooner after 24 h only.

Increasing the concentration of glycerol from 3 to 6 and 9% did not affect the motility during first 24 h, but during subsequent storage (48 h) 6% glycerol was better in milk. Glycerol penetrates sperm cell memberane slowly, therefore, in the absence of yolk (due to non coating of sperm cell membrane) glycerol might have easily entered the sperm cell and exhibited its deleterious effect by inhibiting the motility. In the milk, however, due to presence of fats and total solids this action of glycerol might have been prevented.

Glucose, xylose and raffinose in milk, decreased the motility but fructose, sucrose and table sugar either maintained motility at par or increased it. In tris glucose and xylose could not maintain good motility beyond, 24 h. In citrate sucrose and fructose with 3% glycerol, raffinose and sucrose with 6% glycerol and raffinose and fructose with 9% glycerol markedly increased the motility but only up to 24 h of preservation. In the tris 48 h after storage 30% and above motility was recorded in combination with 3% glycerol containing either of 2% glucose, 1.0, 1.5 or 2.0 raffinose. 1.5 and 2.0% fructose and 2% table sugar. Motility in other combinations of 3% glycerol was less than 30%. And after 72 h none of the combination of 3% glycerol could provide 10% motility except raffinose. The situation was more poor with combinations containing 6 or 9% glycerol and in none of the combinations motility could exceed 20%, after 48h and 10%, after 72 h except few combinations having raffinose and sucrose. In the citrate combinations, however 6 and 9%, glycerol was severly detrimental and in none of combinations 20% or above motility was achievable after 48 h storage. In majority of combinations motility declined to about 10 or 15% after 48 h and almost zero which 72 h except for raffinose combination where it was 25% (48 h) and 10% (72 h).

The protective action of sugar is influenced by tonicity of dilutor, presence of other protective agent in the dilutor and molecular weight of sugar. Sugars when present on either side of cell membrane protect the cell. Better motility with, sucrose and raffinose, particularly in milk might be due to their extracellular protection. Better motility for longer duration after sugar addition in milk might be due to some synergestic and complimentary effect of fat, protein, lactose and total solids present in milk. (Prakash and Dubey, 1985).

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Effect of Cryopreservation on Sperms of Murrah Bulls

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ABSTRACT

The effect of cryopreservation on seminal characteristics was studied on 72 ejaculates from 12 breeding Murrah bulls (6 ejaculates/bull) belonging to the State Frozen Semen Bank, Bhopal. On cryopreservation the sperm abnormalities remained unaltered, whereas, a significant drop (P<0.01) in the semen, pH, sperm motility, live sperms and their biometry was observed.

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Since last decade the frozen semen of Murrah bulls is in use throughout the country with a objective to improve the milk production rapidly by exploiting the superior germplasm. The success of breeding programme using frozen semen for artificial insemination has highly variable results in buffaloes. Hence, accurate evaluation of the semen soon after collection and freezing is necessary as its lower fertility potential brings the artificial insemination to a disrepute.

MATERIALS AND METHODS

A total of 72 ejaculates from 12 healthy breeding Murrah bulls (6 ejaculates/bull) located at the State frozen semen bank, Bhopal, were collected during morning hours once a week from each bull by artificial vagina technique (Salisbury *et. al.*, 1985). Its voluem, pH (indicator strips), sperm motility (Zemjanis, 1970), concentration (Salisbury *et. al.*, 1985), live count, abnormalities (Blom, 1971) and biometry (Deka and Rao, 1987) were studied. The tris egg yolk citrate was used for semen dilution. The samples were kept for half an hour at room temperature for glycerolizaiton and then filled in 0.25 ml capacity mini straws and frozen by the standard protocol (Willadsen, 1979). For forzen semen evaluation, thawing of straw was done at 40°C temperature for one minute. The spermatozoal post-thaw motility, live per cent, abnormal per cent and biometry were studied on 7th day post freezing.

RESULTS AND DISCUSSION

The mean ejaculate volume was 2.52 ± 0.19 ml, which differed significantly (P<0.01) from bull to bull. This may be due to the functions of accessary sex glands which are androgen dependent. The mean initial seminal pH was 6.76± 0.02 which dropped significantly (P<0.01) to 6.50 ± 0.02 post-freezing. Nath *et. al.*, (1991) described that the decline in pH value was due to accumulation of lactic acid, a metabolic byproduct of sperm in the semen. The inidividual motility decreased significantly (P<0.01) from 78.54 ± 0.03% in neat semen to 46.17 ± 0.86% in the frozen semen which may be due to the acids arising from anaerobic sperm metabolism inhibiting the sperm motility.

The mean sperm concentration in the breeding Murrah bull semen was 1311.18 ± 38.36 million/ml which differed significantly (P<0.01) between the bulls. The live sperm dropped significantly (P<0.01) from 85.00 ± 0.83% in neat semen to 53.74 ± 1.19% in frozen semen

which may be conclusive in assessing the relative fertility of the bull. There was no significant difference in the major or total sperm abnormalities pre or post freezing. These abnormalities usually increase significantly due to faulty handling and cryopreservation technique (Salisbury 1985).

The sperm dimensions (length/width) decreased significantly ($P<0.01$) due to freezing and thawing. This reduction in sperm head size may be due to shrinkage of acrosome during freezing (Mukherjee and Dott, 1960) and injury to sperm cells resulting into leakage of certain protein material from the sperms into the extracellular media (Mann and Mann, 1981). Similarly, the midpiece, tail and total sperm length also decreased significantly ($P<0.01$) due to freezing and thawing of the semen. Hence, it is concluded that the cryopreservation of the Murrah semen of the State frozen semen bank did not cause an increase in sperm abnormalities. However, the effect on the semen pH, and on the sperm motility, live number and dimensions was within the permissible limits for the relative fertility.

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Effect of Egg Yolk on Ultrastructure of Head of Ram and Boar Spermatozoa During Cold Shock

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ABSTRACT

A total of 12 ejaculates, 6 from each Finnish Landrace ram and Landrace boar were used in the present study to assess the effect of egg yolk on ultrastructure of ram and boar spermatozoa during cold shock. All the samples were fixed for transmission electron microscopy and the ultrathin section (50-80nm) were examined in a JEOL 1200 Ex electron microscope. The percentage of sperm with loss of plasma membrane, acrosomal content and damaged acrosomal membrane were 74.67 ± 1.10 and 75.67 ± 1.10 in cold shocked spermatozoa without egg yolk and 37.5 ± 0.94 and 62.67 ± 0.75 in cold shocked spermatozoa with egg yolk in ram and boar respectively. Egg yolk has a protective effect on ram spermatozoa during cold shock but not on boar spermatozoa.

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The spermatozoa are sensitive to rapid cooling (cold shock). This cold shock causes extensive sperm membrane damage leading to irreversible loss of motility and fertility (Watson, 1981). Egg yolk is seen to provide protection to ram and bull spermatozoa against cold shock (Blackshaw, 1981). Egg yolk does not appear to offer much protection for boar spermatozoa (Benson *et. al.,* 1967; Pursel *et. al.,* 1973) inspite of earlier claims (Lasley and Bogart, 1944 and Polge,1956). However, the extent and site of damage on the ultrastructure are still conflicting. The present study was made to assess the effect of egg yolk on ultrastructure of ram and boar spermatozoa during cold shock.

MATERIALS AND METHODS

A total of 12 ejaculates, 6 from each Finnish Landrace rams and landrace boar, were collected once in a week for the present study. Motility was verified using a Hamilton Thorn motility analizer and the concentration was determined with previously calibrated colorimeter. The experiment was set diluting each sample into four as follows:-

1. in Hepes glucose saline (Robertson and Watson, 1987)
2. in Hepes glucose saline with 3% egg yolk
3. in Hepes glucose saline and cold shocked
4. in Hepes glucose saline with 3% egg yolk and cold shocked

0.2 ml semen was pipetted into 0.2ml diluent. For cold shock, samples were put into cracked ice for 5 minutes. All the samples were fixed for transmission electron microscopy as per the method described by Plummer and Watson (1988). Ultrathin section were cut using glass knives on a Reichart Ultramicrotome. The silver grey ribbons were mounted on 200 mesh hexagonal grids. Sections were stained twice using uranyl acetate for 6 minutes and lead

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citrate for 4 minutes. The section were examined in electron microscope. At least 200 sperm head were counted from each treatment and the results were averaged in percentage for each group. The sperm head were classified as per Plummer and Watson (1988) with modification as follows

1. Intact spermatozoa with intact plasma membrane and intact acrosome (Fig-1)
2. Plasma membrane present but either dilated or broken (Fig-2)
3. Plasma membrane absent acrosome intact with dark homogenous content (Fig-3)
4. Plasma membrane absent. loss of acrosomal content, acrosomal membrane wavy or damaged (Fig-4)
5. Plasma membrane present, loss of acrosomal content (Fig-5)

RESULTS AND DISCUSSION

The loss of plasma membrane and damage of acrosome were minimum and similar in the treatment 1 and 2 for ram and boar. The percentage of sperm with loss of plasma membrane, acrosomal content and damaged acrosomal membrane were 74.76 ± 1.10 and 75.67 ± 1.10 in ram and boar respectively in cold shocked spermatozoa without egg yolk (treatment 3). The acrosome showed swelling and the outer acrosomal membrane was convoluted and its smooth contour was lost with simultaneous increase in loss of homogeneousity of the acrosomal content. The percentage of spermatozoa with intact plasma membrane and intact acrosome was higher (18.33±0.87) in ram than in boar (10.83±0.89) and the percentage of spermatozoa with mild damage of plasma membrane (dilated or broken) was with egg yolk (Treatment 3 and 4). On the otherhand the loss of plasma membrane and acrosomal content and damaged acrosome was higher in boar (61.67±0.75) than in ram (37.5±0.94) in cold shocked spermatozoa with egg yolk.

The higher damage of boar spermatozoa than ram sprmatozoa after cold shock in extender containing egg yolk might be due to lesser protective action of egg yolk against cold shock for boar spermatozoa than for ram spermatozoa. Lecithin, the active fraction of egg yolk was reported to protect bull and ram spermatozoa against cold shock (Quinn *et. al.*, 1980; Watson, 1981) but was apparently without benefit to boar spermatozoa (Benson *et. al.*, 1967; Pursel *et. al.*, 1973). The structures frequently damaged by cold shock were the plasma membrane and the acrosome recorded in the present study was in agreement with Watson (1981).

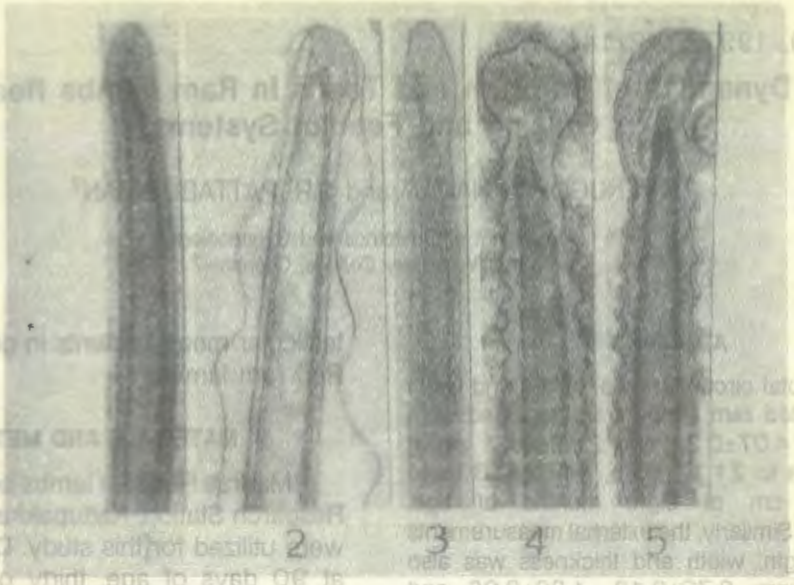

Fig 1. Intact spermatozoa with intact plasma membrane and intact acrosome.
2. Plasma membrane present but either dilated or broken.
3. Plasma membrane absent acrosome intact with dark homogenous content.
4. Plasma membrane absent, loss of acrosomal contents, acrosomal membrane wavy or damaged.
5. Plasma membrane present, loss of acrosomal content.

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Growth Dynamics of Scrotum and Testis in Ram Lambs Reared Under Grazing and Feedlot Systems

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ABSTRACT

The scrotal circumference, height and width in Madras Red ram lambs was increased from 7.56±0.36, 4.07±0.21 and 2.62±0.11 cm at three months to 21.12±0.55, 11.62±0.30 and 8.16±0.17 cm at eight months of age, respectively. Similarly, the external measurements of testis length, width and thickness was also increased from 2.73±0.12, 1.20±0.06 and 0.83±0.06 cm at three months to 7.80±0.18, 4.09±0.09 and 3.60±0.10 cm at eight months of age, respecitively. Significant differences were observed between age groups in all scrotal and external testicular measurements. The overall means of scrotal and testicular measurements were significantly higher in ram lambs under feedlot system than in ram lambs under grazing system.

———x———x———

Scrotal size especially scrotal circumference provide a basis for selection for breeding rams of all ages. Measurements of testicular size may be used as a selection criteria in identifying superior males in the first year of postnatal period (Lucas *et. al.*, 1983).

Rearing ram lambs by feeding concentrates is being adopted in All India Co-ordinated Sheep Projects with the main objective to achieve the desired body weight and carcass quality at a predetermined postnatal age. No systemic study have been carriedout in such feedlot system on postnatal developement of reproductive organs. Hence, an investigation was carriedout to study the effect of grazing and feedlot systems on external scrotal and testicular measurements in growing Madras Red ram lambs.

MATERIALS AND METHODS

Madras Red ram lambs born at livestock Research Station, Kattupakkam, Tamil Nadu were utilized for this study. During weaning at 90 days of age, thirty one ram lambs of uniform body weights were selected and randomly divided into two groups of different feeding systems namely grazing (Seventeen ram lambs) and feedlot (Fourteen ram lambs) systems. The ram lambs in grazing system were sent for grazing daily. Concentrate feed calculated at the rate of 100g per lamb from 3 to 5 months of age and 200g per lamb from 5 to 8 months of age was fed in bulk for all ram lambs. Whereas, ram lambs in-feedlot system were housed in individual wooden partitioned pens and fed adlibitum individually round the clock from 3 to 8 months of age. The ram lambs in feedlot system were fed with pelleted feed comprising of 50 per cent lucerne meal plus 50 cer cent concentrate.

The scrotal and testicular measurements were recorded externally from 3 to 8 months of age at 15 days

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interval as per the methods described by Shrestha *et. al.,* (1983) and Sutama and Edey (1985). The number of ram lambs utilized for the study of external scrotal and testicular measurements were declined at the rate of two lambs per month in each group due to utilization of them for some other investigations. The data collected were analysed as per least square analysis of Harvey (1975).

RESULTS AND DISCUSSION

The scrotal circumference recorded in the present investigation was 7.56±0.36, 9.45±0.37, 12.68±0.40, 17.15±0.43, 19.70±0.47 and 21.12±0.55 cm at 3,4,5,6,7 and 8 months of age, respectively. Kumi-Diaka *et. al.,* (1985) reported the scrotal circumference in ram lambs at 6 months (14.5 to 18.5 cm) and 8 months (15.2 to 20.1 cm) which were comparable with the results in this study. However, Shrestha *et. al.,* (1983) recorded higher values of scrotal circumference in exotic breeds. This may be due to higher body size in these breeds than Madras Red ram lambs. In the present study, the average daily gain in scrotal circumference of ram lambs at 3-4, 4-5, 5-6, 6-7 and 7-8 months was 0,63, 0.88, 1.03, 0.72 and 0.31mm in grazing and 0.68, 1.31, 2.07, 1.12 and 0.50mm in feedlot systems respectively. The gain in scrotal circumference was gradually increased from 3 to 6 months and then decreased from 6 to 8 months of age. Ley *et. al.,* (1990) also noticed a rapid growth from two to six months of age in exotic rams. The increase in the scrotal circumference at a particular age during the postnatal period might be due to the physiological changes in the testicular parenchyma. The scrotal circumference was more correlated with the body weight (0.99) than with age (0.93). Foster *et. al.,* (1989) observed positive and highly significant correlation between scrotal circumference and testicular weight in rams.

The scrotal height in ram lambs increased from 4.07±0.21cm at 3 months to 11.62±0.32 cm at 8 months of age. The scrotal width also incresed from 2.62±0.11 cm at 3 months to 8.16±0.17cm at 8 months age. There is no comparable information available in literature regarding scrotal height and width in growing ram lambs.

The length, width and thickness of the testis in ram lambs during postnatal period was increased from 2.73±0.12, 1.20±0.06 and 0.83±0.66cm at 3 months to 7.80±0.18, 4.09±0.09 and 3.60±0.10 cm at 8 months of age. There was not much published work in the literature on the testicular measurements of growing ram lambs in Indian breeds for comparison. Louda and Stolc (1981), Shrestha *et. al.,* (1983) and Lunstra and Echternkamp (1988) reported higher testicular measurements in exotic and crossbred rams than in Madras in the present study.

Highly significant difference was recorded between grazing and feedlot systems in scrotal and testicular measurements in this study. The overall mean of scrotal circumference (15.99±0.19cm Vs 13.36±0.17cm), scrotal height (8.27±0.11 cm Vs 6.58±0.10cm), Scrotal width (5.77±0.06cm Vs 5.10±0.05 cm), testis length (5.81±0.06 cm Vs 4.96±0.06 cm), testis width (2.91±0.03cm Vs 2.48±0.03 cm) and testis thickness (2.51±0.03 cm Vs 2.07±0.03 cm) was significantly higher in feedlot system than in grazing system. In ram lambs under grazing, the scrotal and testicular measurements were lower due to nutritional deficiency and low energy intake which is in agreement with Row and Murray (1984) who stated that for testicular growth

metabolizable energy intake was important. Kumi-Diaka *et. al.,* (1985) observed differences in scrotal circumference between extensively and intensively managed Yankasa ram lambs. Significantly higher overall means for all the parameters could be attributable for physiological growth of the testicular paranchymatous tissue due to increased feeding level. Martin *et. al.,* (1987) mentioned that the nutritional supplementation stimulated testicular growth in rams due to primarily an increae in size of the seminiferous tubules. It is observed that by and large, all external measurements of scrotum and testes followed similar trend upto 4-5 months of age and therafter differed signficantly between grazing and feedlot systems. Similarly Cameron *et. al.,* (1988) observed that the testicular size increased in the rams on the high plane of nutrition than the submaintenance ration and differed significantly by the end of nine weeks of treatment.

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Histochemical Activity of Phosphatases and Lipid Content of Epididymis in Indigenous and Crossbred Pigs

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ABSTRACT

This study was undertaken to know the histochemical activity of phosphatases and lipid content of epididymis in indigenous and crossbred pigs aged from '0' days (at birth) to 8 months. In both the breeds, regional differences (caput, corpus and cauda epididymis) were observed in the activity of phosphatases and lipid content. The phosphatases activity appeared as black granules in the cytoplasm of epithelial cells (ACP) and sub-epithelial connective tissue as well as in apical portions of epithelium (ALP). The spermatozoa in the tubular lumen showed slight ALP activity. The lipid content was noticed in the luminal sides of the epithelium and it was more in crossbred than indigenous pigs.

Information on the phosphatases (Acid phosphatase (ACP) and alkaline phosphatase (ALP) activity and lipid content in the indigenous pigs is meagre. Hence, the present work was undertaken to investigate the activity of these in the indigenous pigs and compare these with crossbred pigs.

MATERIALS AND METHODS

Epididymis from 60 male pigs (30 each of indigenous and crossbred (indigenous 25% x Large White Yorkshire 75%) aged from '0' day (at birth) to 8 months maintained at All India Coordinated Research Project on Pigs, College of Veterinary Science, Tirupati, were collected. Epididymis was arbitrarily divided into 3 regions viz., caput, corpus and cauda. Tissues pieces from these regions were collected and fixed in chilled acetone, cold calcium formaline and 10% neutral formaline for the demonstration of phosphatases (ACP and ALP) activity and lipid content respectively. Frozen sections of 8-10u thick were cut and stained with Gomari (1952), and oil red-o-methods (Humason, 1972) respectively. After staining histochemical localization and intensity of ACP, ALP and lipid content were studied, graded and compared between breeds.

RESULTS AND DISCUSSION

Phosphatases: In both the breeds phosphatases activity appeared as black granules in the cytoplasm of epithelial cells especially towards the luminal borders (ACP) and in the subepithelial connective tissue as well as in apical portion of the epithelium (ALP). Similar to the observations in this study Rollison (1955) and Russel and Stallcup (1967) in bulls indicated ACP activity in the basal cytoplasm of lining epithelium and in supranuclear location, respectively. But Wrobel and Fallenbacher (1974) observed ACP activity in the golgi region of the ciliated cells in pigs.

The ACP and ALP activity was found to be in traces upto 3 months and moderate during 4 to 6 months in all the segments of epididymis. But during 7 and 8 months the ACP activity was marked in corpus and moderate in caput and cauda and the ALP activity was moderate in caput and corpus

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and marked in cauda. This difference in the activity of phosphatases at different regions of epididymis was also reported by Wrobel and Fallenbacher (1974) in pigs and Sharma *et. al.,* (1986) in goats. The ALP activity was also noticed in the spermatozoa present in the epididymal lumen. Similarly Renu Arora *et. al.,* (1977) noticed ALP activity in the spermatozoa of Rhesus monkeys.

The presence of a higher activity of phosphatases at the cellular apex in the corpus (ACP) and cauda (ALP) epididymis might be due to its involvement in the absorption and transfer of organic molecules across the cell membranes of these regions (Renu Arora *et. al.,* 1977). Similarly the higher activity in the spermatozoa indicates phosphatase transfer rather than hydrolysis due to presence of high phosphokinases of transphorylases. In this investigation, the ALP activity was higher compared to ACP activity. Sharma *et. al.,* (1986) also reported a higher ALP activity in their studies.

Lipids: In both the breeds the lipid content was in traces in the luminal side of the epithelial cells in all the regions upto 4th month. It was mild in caput and corpus and moderate in cauda from 5 to 8 months in indigenous pigs. In crossbred pigs the lipid content was moderate in all regions of epididymis from 5 to 8 months. This increase in the lipid content from 5th month onwards in all regions in crossbred pigs was also reported by Chandrapal and Bharadwaj (1981) in their studies in buffaloes. This higher lipid content in crossbred pig probably be due to their higher body weights during that particular age when compared to that of indigenous pigs at puberty.

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Seminology as an indicator of epididymal Dysfunction in Bulls

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The volume of the ejaculate and the sperm concentration are subject to considerable physiological variations from one day to another, to variations from one individual to another and also with age. Repeated low values, however, can be an indication of disturbed sexual function in bull.

Another very important indication of abnormal sexual function is impaired or abnormal sperm motility, which is mostly secondary to damages of the testicules with disturbance of the spermatogenesis or spermateliosis. In investigations by Gustafson (1966) and Gustafson et al (1972 & 1974) Rao (1976) and Moulikrishna and Rao (1986) it was shown that disturbances of the sperm motility in combination with changes of the tail morphology could be an indication of epididymal dysfunction in bulls and buffaloes. Admixture of inflammatory exudate from infections of the accessory sexual organs can also impede sperm motility. Finally specific changes of the kinetic structure of the sperm tail have been described as the cause of motility disturbances.

The origin of pathological sperms:

Already in the beginning of the century there were indications of a correlation between the number of pathological aperm in the ejaculate and the fertility of the male animal. It was mainly Williams and co-workers at Cornell University in U.S.A. who were the pioneers and after extensive investigation maintained that about 17% pathological sperm was the limit between normal and infertile bulls (Williams and Savage, 1927). Lagerlof (1934) made a very important contribution to the understanding of the origin and importance of pathological sperms. Through simultaneous examinations of the number of pathological sperm in the ejaculate and the histology of the testicles in bulls with impaired fertility he could show that there was a correlation between pathological changes of the germinal epithelium and the number of pathological sperm in the ejaculate. Lagerlof's conclusions have been confirmed by many later investigations. It has also been shown that not all pathological sperm changes have a testicular origin. As mentioned earlier it has been shown that epididymal dysfunction can have an effect on sperm morphology, mainly causing characteristic bending and coiling of the sperm tails.

Pathological sperm in the duct system:

Rao (1971) examined in depth the pathological sperms in the duct system from ductuli efferentes to the ejaculate. He could show that both in normal and infertile bulls the composition of the sperm population leaving the testicle undergoes dramatic changes during the passage through epididymis. There is a connection between degeneration of the geminal epithelium and the number of pathological sperm in ductuli efferentes. In normal bulls in efferent ductules there are 58 abnormal sperm

heads of 500 while in bulls with marked testicular degeneration there are 183 abnormal sperm heads of 500 counted. During the passage through the epidiymis these frequencies change. The number of abnormal sperm in the ejaculate of normal bulls and in the bulls with marked testicular degeneration are 46 and 107 abnormal sperms of 500 counted respectively. Indipendent of the degree of degeneration the numbers of pathological sperm decrease on the way to the ejaculate. The decrease is much more pronounced in bulls with testicular degeneration than in normal bulls. The diappearance of abnormal sperm is selective and affects mainly microsperm and sperm which are pear shaped, narrow at the base and undeveloped.

The frequency of abnormal sperm tails undergo important changes during the passage through the duct system. The frequencies are low in ductuli efferentes with a possible tendency to increase with increasing degree of testicular degeneration. The number of abnormal sperm tails per 200 sperms was 0.92 and 10.92 in the efferent ductules and ejaculate respectively in the bulls with marked testicular degeneration.

The frequency of proximal cytoplasmic droplets is almost 100% when the sperm leave the testicle. The number decreases during the passage through the epididymis to about 6% while in bulls with marked testicular degeneration there are still about 40% proximal droplets in the ejaculate. This means that the disturbance of the maturing process in the epididymis in bulls with testicular degeneration may be influenced by factors present already in the germinal epithelium.

This investigation lead to the following conclusions. Examination of sperm morphology in combination with other laboratory tests is a valuable tool in the estimation of the potential fertility of a bull. A frequency of pathological sperm above the physiological limits of 17-18% is an indication of disturbances of the spermatogenesis/spermateliosis or sometimes epididymal dysfunction and gives most probably a lowered fertility. Repeated examinations facilitate the prognosis. A frequency of abnormal sperm within physiological limits is a prerequisite but no guarantee for normal fertility.

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Glycocalyx of Ram Spermatozoa - A High Resolution Electron Microscopic Study

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A rich polysaccharide layer or glycocalyx is present on the surface plasma membrane of spermatozoa. It has been investigated with the use of a variety of electron microscopic cytochemical study (Jauregui, 1975). The glycocalyx plays an important role in certain physiological process like capacitation, acrosome reaction and fertilization.

The present study was undertaken to observe the glycocalyx of ram spermatozoa using alcian blue and lanthanum.

MATERIALS AND METHODS

A total of 4 ejaculates from Finnish Landrace rams maintained at Royal Veterinary College, London were collected once in a week using standard artificial vagina. The samples were fixed in a mixture of 2% glutaraldehyde in 200mm cacodylate of 290m osm, pH 7.4 with 0.5% alcian blue (with and without 0.05m magnesium chloride) and postfixed in 1% osmium tetraoxide with 1% lanthanum nitrate in 200mM cacodylate buffer. Control semen samples were treated without the use of alcian blue and lanthanum. Samples were fixed for transmission electron microscopy as per the method described by Plummer and Watson (1988).

RESULTS AND DISCUSSION

Electron microscopic examination of the spermatozoa stained with alcian blue and lanthanum nitrate revealed electron dense deposites on the surface of the plasma membrane (Fig.1). The increase in staining was observed where magnesium chloride was incorporated. The staining characteristics appeared as continunous or discontionuous granules confined to the outer surface of the plasma membrane. The intensity of electron opaque materials at the surface membrane varied within the same sample. Control samples not treated with stain did not exhibit the electron dense coating.

Fig. Electron dense deposits on the surface of the plasma membrane of sperm head.

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Monocentric Testicular Cyst in Buffalo Bull

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There appears to be no information available on the occurence of testicular cyst among domestic animals and buffalo in available literature. However conversely there is no paucity of cysts in various other organs which are usually inert. This paper is to place on record the occurence of testicular cyst in a non-descript buffalo bull.

During investigation of andrological abnormalities a case of testicular cyst was recorded unilaterally in a nondescript buffalo bull from local slaughter house. On screening, the right testis was viewec comparatively larger and almost assumed on oval appearance than contralateral testis. The incision divulged a centrally located cavity containing clear watery sap which easily flow out (Fig.). The cyst was appeared to be of non-parasitic origin as there was no evidence of parasitic scolices submerged in the fluidy contents. The tissue was collected in ten percent neutral formalin for histopathological examination.

Histologically, tissue section revealed the cyst lined by a thin connective tissue stroma abutting the atrophied seminiferous tubules, whereas distantly placed seminiferous tabules depicted compensatory dilatation. The inflammatory cell response was absent both in and outside the cystic space.

The genesis of cystic rete testis has been reported in cat apparently to be caused by congenital lack of communication between efferent duct and the epididymis (Jubb *et. al.,* 1993). Other reasoning could be derived analogousely to the origination of the prostatic and other variety of cysts, which might be resulted due to alteration in the glandular epithelial morphology and secretion. (Allen and others 1991, Cheville 1993). Further there may be a possibility of excessive oedematous fluid accumulation on account of pathological obliteration of lymphatic duct.

Incised buffalo testis showing centrally located cyst

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Serum Testosterone Profile following Foot and Mouth and Black - quarter vaccination in Murrah Bulls*

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Vaccination against diseases which are performed as a routine in bull station leads to a major stress factor affecting testicular funtion. Cellular damage following vaccination (Rao and Venkataswami, 1971) and subsequent effect of quantity, quality and preservability of semen (Saxena *et. al.,* 1976) have been reported. However, no information is available regarding the effect of vaccination stress on serum testosterone concentration. Hence the present investigation was undertaken to study the testosterone production by the Leydig cells following FMD and BQ vaccination in Murrah buffalo bulls.

MATERIALS AND METHODS

Six healthy Murrah buffalo bulls of same age group (5-8 years) maintained at Buffalo Frozen Semen Station, Erode were randomly selected and utilized in this study. One group of three bulls was vaccinated with FMD vaccine and other group of three bulls with BQ vaccine. Blood collected four times prior to vaccination were used as control. After vaccination, blood samples were collected every Tuesday and Friday for first two weeks and then restricted to once a week upto 5th week post-vaccination. The bulls were administered GnRH analogue Buserelin (Hoechst, India) intramuscularly at a dose rate of 4ng/kg body weight. Blood samples were collected from jugular vein 150 minutes post GnRH injection. The blood samples were allowed to clot overnight at 5°C and were centrifuged for serum separation. The separated serum samples were stored at -20°C till the testosterone assay. Serum testosterone was estimated using Coat - A - Count Total testosterone kit supplied by Diagnostic Products Corporation, USA. The kit was a solid-phase I^{125} radio immuno-assay designed for the quantitative measurement to testosterone in serum. The results were statistically analysed using methods of Snedecor and Cochram (1967).

RESULTS AND DISCUSSION

The Prevaccination level of 2.32+0.45 ng/ml in FMD group and 2.57±0.52 ng/ml in BQ group remained almost the same through out the post vaccination period from 4 to 39 days. The mean values of serum testosterone after vaccination were not different statistically ($P>0.05$) from the pre-vaccination mean value. Similar observation was done by Milton *et. al.,* (1991) following exposure to elevated ambient temperature in Angus bulls. They also suggested that heat stressed bulls are capable of responding to endogenous LH stimulation by secreting testosterone. Robers (1986) reported that heat has no effect on the Leydig cells physiology.

However, Rhynes and Ewing (1973) observed decrease in plasma testosterone following exposure to heat stress. These

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workers used an infrequent sampling schedule which may not have allowed an accurate estimation of plasma testosterone level due to episodic pattern of testosterone secretion in bulls (Katongole *et. al.,* 1971).

From the present investigation, it can be concluded that Leydig cells under vaccine stress are capable of responding to GnRH medicated LH by secreting testosterone.

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Repeatability of Semen production traits in Jersey Bulls*

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Studies on genetic variability on semen production traits were started as early as 1950s (Lasley, 1951). However, much work has not been done in using the same with other non-genetic effects for evaluation of semen production in breeding bulls. Very little emphasis is given towards this aspect of studies on exotic bulls maintained under Indian management conditions. Hence this investigation on the repeatability of semen production traits of Jersey bulls was undertaken.

MATERIALS AND METHODS

Data on semen production traits accrued over a period of four years were collected from Jersey bulls maintained in an organized stud farm at Udagamandalam, The Nilgiris. Ejaculate volume, Initial motility and Sperm concentration (for first and second ejaculates), Total extended volume, pre-freeze motility, post-thaw motility and total doses of frozen-semen per collection were the semen production traits studied. The repeatability for semen production traits was estimated by using the following statistical model and formula.

statistical model:

$$y_{km} = \mu + \alpha_k + e_{km}$$

where y_{km} is the m^{th}th observation on the k^{th}th individual.

$$\text{repeatability (R)} = \frac{\sigma^2 w}{\sigma^2 w + \sigma^2 e}$$

$$SE(R) \simeq \sqrt{\frac{2(n.-1)(1-R)^2\,[1+(k_1-1)R]^2}{k_1\,(n.-N)(N-1)}}$$

The repeatability estimates of semen production traits in this study ranged from 0.03 for second ejaculate motility and post thaw motility to 0.19 for second ejaculate volume. The repeatability for other traits were also observed to be within this range. These estimates were lower than the values reported by Chandler *et. al.*, (1985), Taylor *et. al.*, (1985) and Stalhammer *et. al.*, (1989). The lower repeatability values obtained in the present study might be due to differences in the population studied and method of estimation. The temporary environment factors appear to have more influence than the permanent differences among bulls. Besides, selection of bulls for semen production might also bring about a downward bias in repeatability.

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Effect of superovulation and nonsurgical Embryo recovery on subsequent reproductive performance in Cross Bred Cattle

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Early resumption of oestrus cyclicity in superovulated donors after embryo recovery has great economical bearing. This would facilitate repeated superovulation (Dinar, 1987) or to breed such animals at the earliest (Chupin *et. al.*, 1984). In the present paper effect of superovulation and nonsurgical embryo recovery on subsequent reproductive performance in cross bred cattle has been reported.

MATERIALS AND METHODS

Fifty six cross bred cows superovulated with FSH-P(30-36 mg) in decreasing dose schedule were used for the present study. Embryos were collected on day 7 by non surgical method. Following non surgical recovery of embroyos, donors were divided into two groups.

Group I : This group consist of 44 animals. They were treated with singgle I/M injection of $PGF_2\alpha$ [25 mg dinoprost tromethamine (Lutalyse)] following non-surgical flushing of embroys.

Group II : This group had 12 animals. They were not treated with $PGF_2\alpha$ following non surgical flushing and served as control.

All the experimental animals following non-surgical recovery of embryos and injection of $PGF_2\alpha$ were observed closely and regularly for the onset of oestrus. Animals confirmed in estrum were inseminated. They were examined for confirmation of pregnancy 45-60 days following insemination.

RESULT AND DISCUSSION

The onset of oestrus following embryo collection was significantly earlier in $PGF_2\alpha$ treated donors than the untreated control. (9.45±2.45 Vs 22.50±2.97). The results also indicated that a higher percentage of animals were in oestrus within 16 days following the embryo recovery than the untreated control (93.16 Vs 33.33%). The delay in the return to oestrus in donors not treated with $PGF_2\alpha$ following nonsurgical embryo recovery has also been reported by Rowe (1980) and Garcia *et. al.*, (1983). The delay in onset of oestrus may be due to higher progesterone concentrations resulting from more than the normal number of corpora lutea (Solti *et. al.*, 1978). Earlier onset of oestrus within 16 to 17 days by majority of donors treated with PGFα of the present study are in agreement to Greve and Lehnjensen (1978) and Shioya *et. al.*, (1985). The progesterone level in $PGF_2\alpha$ treated donor cows has been reported to decline within 5 days after PGFα (Lopez Barbella, 1979). The results of subsequent fertility revealed no difference in conception

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rate and A.I per conception between the $PGF_2\alpha$ treated and untreated control cows. (88.0% Vs 100%; 2.22 Vs 2.20). The interval between flushing and conception was shorter in the $PGF_2\alpha$ treated than the control (44.37±12.03 Vs 57.66±11.34). The results demonstrated that superovulation and non surgical recovery of embryos has no adverse effect on subsequent reproductive ability of donors. Similar findings have also been recorded by Moyeast *et. al.,* (1987). In contrast, some workers, however, have reported lower conception rate and higher number of services per conception following superovulation and non surgical embryo recovery (Greve and Lehnjensen, Moyaest *et. al.,* 1987; Cowen and Sosnik, 1987).

It may be concluded that administration of $PGF_2\alpha$ soon after flushing in superovulated donors brings most of the animals in oestrus early and reduces the flushing to conception interval.

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In-vitro Maturation of different types of Bovine Oocyte

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The cumulus cells layers adhered to the zona pellucida play an important role in achieving *in-vitro* maturation of oocyte (Martiono *et al.*, 1992; Mano and Kuwayama, 1993). The present investigation was made to study the effect of different types (based on layers of cumulus cells) of bovine oocyte on *in-vitro* maturation.

MATERIALS AND METHODS

The cattle ovaries obtained from abattoir were washed thoroughly in tap water followed by warm physiological saline solution (37°C) and Dulbecco's phosphate buffered saline (PBS). Oocytes were collected from the follicles by both aspiration and dissection techniques. With the help of a 20 guage needle and a 2ml syringe containing 1ml of PBS + 10% heat inactivated cow serum (HICS).

In the dissection technique the follicles (of 2 to 5mm diam) were dissected out from the ovary with the help of scissors and scalpel. Then in a petridish containing PBS+10% HICS oocytes were taken out from the follicles by rupturing them under a steriozoom microscope. Finally, oocytes were transferred into the embryo collection dish containing PBS + 10% HICS. the cumulus-oocyte-complexes (COC) were classified based on layers of cumulus cells surrounding the zona pellucida into type A-having 3 or more complete layers, B-1 to 2 complete layers, C-incomplete layers of cumulus cells and D-without any cumulus cells or nude oocyte. The type A, B and C oocytes were *in-vitro* cultured in TCM-199 at 39°C in an atmosphere of approximately 5% CO in air for 24 hours. The statistical analysis of the data was made as per Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The recovery of type A, B, C and D oocytes was found to be 1.85±0.15 and 3.00±0.23; 0.71±0.08 and 0.99±0.09; 0.58±0.07 and 0.63±0.08 and 0.45±0.06 and 0.28±0.05 respectively in both aspiration and dissection techniques. This is in accordance with the findings of Leibfried and First (1979) and Hanada *et al.*, (1986). The higher incidence of recovery of type A oocyte indicated tightly adhering of cumulus cell layers surrounding the zona pellucida in most of the oocytes. There was significantly higher recovery rate of A ($P<0.01$) and B ($P<0.05$) type oocytes in dissection technique than in aspiration technique. This concurs with the findings of Wahid *et al.*, (1992). The recovery rate of type D oocyte with the findings was significantly ($P<0.05$) higher in aspiration technique than in dissection technique. This might be due to the fact that aspiration of follicular oocyte and subsequent forceful expiration using needle and syringe might cause detachment of loosely adhered cumulus cells from the oocyte.

The incidence of Mataphase I and Metaphase II was found to be 24.24, 27.03

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and 33.33 and 46.46, 37.84 and 33.33 percent in type A, B and C type oocytes respectively when cultured in medium TCM 199 at 39° for 24 hrs. The incidence of Metaphase II recorded in this study is comparable to that recorded by Fukui *et. al.*, (1982). Although the rate of *in-vitro* maturation (Metaphase II) did not suffer significantly between different types of oocyte it was apparently higher for A type. This findings are in agreement with the findings of Lorenze *et. al.*, (1993).

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Vitrification of buffalo oocytes

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Vitrification of immature cattle oocytes has been attempted with variable success rates (Faku *et. al.,* 1994). However, similar attempts in buffalo have not been made. The present study was undertaken to investigate the suitability of DMSO, Ethylene, Glycol (EG) and Propylene Glycol (PG) as cryoprotective agent during vitrification of immature buffalo oocytes.

MATERIALS AND METHODS

Compact cumulus oocyte complexes collected from ovaries procured from local abattoir were exposed to the cryoprotective agent (DMSO/EG/PG) in gradually increasing concentrations from 0.6M to 2.4M for 3 min each before placing them in 3 M concentration of the cryoprotectant in PBS supplemented with 0.25M sucrose and 4% BSA. Oocytes in final vitrification mixtures were loaded in 0.25ml straws (2 oocytes per straw) and plunged into liquid nitrogen. Straws were removed from at 10, 20 and 30±1 hr post vitrification and thawed at 30°C in water bath for 20 sec. The dilution and removal of cryoprotectant was done in reverse order to that of its addition. Oocytes were washed twice and then incubated in TCM 199 enriched with 10% FCS (supplemented with ovine LH 10 μg/ml, ovine FSH 1 μg/ml and Estradiol-17°β 1 μg/ml) in 5% CO_2 at 39°C for 24 hrs and were fertilized in vitro with frozen-thawed, capacitated buffalo spermatozoa.

RESULTS AND DISCUSSIONS

The maturation rates 34.0, 43.23 and 32.62 per cent of oocytes in the three cryoprotectant groups Gr. I, II and III respectively did not differ significantly and were in agreement to the rate reported for immature mouse oocytes in presence of EG by Miyake *et. al.,* (1993). However, these were higher than values reported by Fuku *et. al.,* (1992) for vitrified bovine oocytes. In the control groups all oocytes were damaged during vitrification.

The fertilization rate of vitrified-IVM oocytes in GrII (26.8%) was little higher than 21.8% reported by Lim *et. al.,* (1992) using 1 M Glycerol on immature bovine oocytes. However, Herrler *et. al.,* (1991) have reported fertilization rate of 42.9% for GV stage bovine oocytes with 2M DMSO and 17.8%lk with 2M PG. The fertilization rate observed in Gr I and III were 15.38 and 15.21 per cent respectively. Fertilization rates observed in the present study were also lower than that reported for IVM-vitrified cattle oocytes (38.3%) by Otoi *et. al.,* (1993) which supports the suggestion of Hamano *et. al.,* (1992) that as a single cell oocyte is likely to be more vulnerable to any environmental challenge than the multicellular preimplementation embryo. Prolonging the storage of vitrified oocytes from 10 to 30 hrs in liquid nitrogen resulted in non-significant reduction in maturation and fertilization rates.

The study indicates the immature buffalo oocytes can be vitrified with reasonable success in presence of Ethylene glycol, DMSO or Propylene glycol.

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Assessment of changes in the Gravid Genitalia of Goats Using Bimanual Palpation

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The present study was carried out to assess palpable changes in the tubular reproductive tract at various stages of gestation so as to diagnose pregnancy and to assess the stage of pregnancy by per-rectal digital palapation combined with abdominal manipulation in normal standing position.

MATERIALS AND METHODS

Fifty two Nanny goats of Kerala Agricultural university Goat farm were examined using Bimanual technique at 28-32 days after artificial insemination and 29 were found to have distension of uterine horns and were properly identified. Among this 20 animals were randomly selected and reexamined at monthly intervals till the date of kidding and the changes palpated in the tubular reproductive tract were recorded.

Nannies were examined in standing position before feeding and watering. Palpation was carried out using the pre-lubricated and gloved index finger of left hand via rectum. Retroversion of tubular tract into the pelvic region was done by the right palm brought upward through the floor of posterior abdomen. Simultaneous and judged manipulation of both right palm and left hand finger enabled assessment of size, shape, consistency, surface characteristics and other changes characteristic to each stage of gestation.

RESULTS AND DISCUSSION

Differentiation of gravid from non-gravid reproductive tract was easily done based on distension from 30 days onwards. Non-pregnant uterus did not show notable distension or asymmetry and in most of the nannies ovaries palpated. Changes noticed in the tubular reproductive tract at various stages of gestation were as follows.

28-45 days: Cervix normal in size. Both uterine horns showed clear distension with fluid filled feeling and located at or just in front of pelvic brim.

45-60 days: Slight tension on the vagina, cervix slightly hypertrophied and soft located at pelvic brim, distended uterus seen in front of the pelvic bone.

60-90 days: Vagina stretched, cervix soft and hypertrophied, uterus within the abdominal cavity and cannot be retorverted into pelvic cavity.

90-120 days: Cervix showed marked hypertrophy and soft consistency and was located at the pelvic brim, uterus will distended, ballotement of foetus was possible and placentomes could be palpated.

120 and above: Placentomes could be palpated on the uterine wall, foetal parts palpable in the pelvic cavity, vagina relaxed and cervix often difficult to palapate.

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Bacterial flora and antibiotic sensitivity pattern of endometritis in cows*

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The present investigation was undertaken to study the bacterial count, bacterial isolates causing endometritis and their antibiotic sensitivity pattern.

MATERIALS AND METHODS

Forty eight cross bred cows with endometritis and eight normal healthy cows brought to the Infertility clinic of Madras Veterinary College were subjected to bacteriological examination. The Uterine discharges were collected by aspiration technique (Steffan *et. al.,* 1984) and samples were charged into the nutrient broth and incubated at 37°C for 24 hours to observe the growth of organisms. Bacterial count was studied from uterine discharges of each cow by adopting plate count technique as described by Malik (1976). Organism were identified on the basis of their morphological, cultural and biochemical characters (Carter 1984). Antiobiotic sensitivity pattern of different bacterial isolates was carried out by standard disc-diffusion technique described by Bauer *et. al.,* (1996). Six antibiotic discs supplied by HI-media Laboratories Ltd., were used, namely Gentamicin (10 mcg), Chloramphenicol (30 mcg) Oxytetracycline (30 mcg), Streptomycin (10 mcg) Ampicillin (10 mcg) and Pencillin (10 units). The interpretation of results was done according to zone size interpretative chart. The data was analysed as per Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The viable bacterial count in uterine discharges of healthy and endometritis cows was 0.0013±0.0008 and 47.0625± 1.1589 (10^6 per ml of uterine fluid) respectively. This was in agreement with the findings of Singla *et. al.,* (1993) who observed bacterial count of 189.39×10^6/ml of uterine fluid in repeat breeder cows.

The predominant isolates in the present study from cows affected with endometritis were Staphylococcus aureas 15 (23.44%), Escherichia coli 13 (20.31%), Corynebacterium pyogenes 12 (18.75%), Streptococcus sps 12 (18.75%), Pseudomonas aeruginosa 9 (14.06%), Proteus vulagaris 2 (3.13%) and klebsiella genitalium 1 (1.56%). This observation was in accordance with the findings of Jacob (1993) and Ambrose and Pattabiraman (1993).

The results of the anitbiotic sensitivity tests revealed maximum sensitivity with Chloramphenicol (87.50%) and least sensitivity with Penicillin (3.13%). Sensitivity of other antibiotics in discending order was

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Gentamicin (70.31%) Oxyteracycline (42.19%) Streptomycin (32.81%) and Ampicillin (10.94%). The findings of present study in respect of antibiotic sensitivity pattern was in agreement with the reports of Venkateswarlu *et. al.,* (1983) Sudhakar *et. al.,* (1986) Khan *et. al.,* (1991), Mohanty *et. al.,* (1992), Jacob (1993) and Sharma *et.al.,* (1993). Cholramphenical and Gentamicin were not routinely used for treatment of uterine infection and hence organisms did not develop the resistance. Most of the isolates were resistant to Ampicillin and Penicillin. This might be due to indiscriminate use of antibotics without resorting to sensitivity tests.

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Therapeutic Efficacy of some Antimicrobial Drugs in repeat breeding cows.

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Repeat breeding syndrome is largely associated with a substantial loss in respect of milk production and calf crop throughout the reproductive life of dairy animals. Most of the time microbial agents as etiological factor causing inflammatory changes in female genital tract resulting in conception failure. Efficacy and merits of uterine treatment with antimicrobial drugs has been studied by many workers (Dholakia *et. al.*, 1987; Sharda *et. al.*, 1991; Krishna*et. al.*, 1994; Singh, 1994). The indiscriminate use of antibiotics has resulted in emergence of resistence in pathogens against antimicrobial drugs. This warrants immediate attention and side by side finding out the scope for the use of less toxic and effective antimicrobial agents in the alleviation of the problem.

In the present study on the basis of *in vitro* studies the effectiveness of antimicrobial drugs were assessed in treating the genital tract infection.

The cervico-vaginal mucus of 108 repeat breeding cows was collected aseptically (Dabas and Maurya, 1988) during estrus phase and were subjected to *in vitro* antibiotic sensitivity test (Cruickshank, 1968). On the basis of *in vitro* sensitivity test intrauterine infusion of the most effective drugs was given for 4 days during the estrus. In the following estrus cervicovaginal mucus was again collected before performing artificial insemination and subjected to microbiological studies to find out the *in vivo* therapeutic efficacy of the drugs. Pregnancy diagnosis by per-rectal palpation was performed on days 45 post A.I.

A total of 58 (53.7%) repeat breeder animals were found having genital tract infection. The various micro-organisms, isolated included Staphylococci, Streptococci, *E. coli* Salmonella sp., Klebesiella spp., Entertobactor spp., Proteus spp. Gram positive bacilli and unidentified Gram negative rods. The culture were found sensitive to different antibiotics. The antibiotic sensitivity was maximum for ciprofloxacin, chloramphenicol, amikacin, gentamycin, kenamycin and norfloxacin. Most of the cultures were found resistant to oxycycline and penicillin.

Chloramphenicol 1 gm plus 20ml distilled water (n=13), gentamycin 400mg plus 20 ml distilled water (n=13), kenamycin 1 gm plus 20 ml distilled water (n=12) and saline water (n=20, control) were infused intrauterine continuously for 4 days during estrus.

Out of 13 animals treated with chloramphenicol, 11(84.6%) animals became infection free in subsequent estrus and 8(72.7%) conceived within 2 subsequent estrous cycles. In the gentamycin treated group, 11(84.6%) animals became infection free and 10(90.9%) conceived within 2 subsequent estrous cycles. In the kenamycin treated group 10(83.3%) became infection free and among these 6(60.1%) conceived. Whereas

in the control group only 2 animals conceived during the study period. Gentamycin and chloramphenicol has been reported to be the most effective antibiotic against bacteria isolated from the repeat breeder (Dholakia *et. al.,* 1987; Sharada *et. al.,* 1991; Krishnan *et. al.,* 1994, Singh and Sekhon, 1995). Therefore, use of conventional treatment without performing *in vitro* senstivity tests is of little value in treating the genital tract infection. And the result of present study indicates that chloramphenicol, gentamycin and kenamycin have good efficacy to get rid off sub-clinical uterine infection and reduce the inter calving period and subsequently economic losses can be reduced to minimum.

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Schistosomus Reflexus in a kid - case report

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Schistosomus reflexus is a non-inherited teratologic defect reported to occur very commonly in cattle, very rarely in sheep and seldom in other species. The condition arises form interruption of specific developmental stages that lead to normal fusion of thoracic and abdominal walls (Potter, 1961).

In the present report the occurence of Schistosomus reflexus in goat was recorded.

A full-term non-descript doe aged 5 years was presented at the Veterinary Dispensary, Ibrahimpatnam, Krishna District (Andhra pradesh) with history of dystokia. Clinical examination per vaginum revealed complete cervical dilatation and presence of four foetal limbs extending into the birth canal. After lubrication by judicious traction, a dead female monstrous kid was delivered with the following features.

The kid was misshapen with complete absence of abdominal wall and all the visceral organs exposed. The liver was enlarged and cystic, the abomasum was distended with fluid. The body and chest walls were bent laterally. The spine was rigid and the head was in juxtaposition with the sacrum. The pelvis was deformed and the limbs were partially ankylosed (Fig.).

The monster was a typical Schistosomus reflexus as per the classification of Arthur *et. al.,* (1989). Bedford (1967) recorded similar case in a goat.

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Monocephalus Dipygus Monster in a Buffalo — A Case report

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Reports on incidence of monstrosities in buffaloes are scarce (Bugalia *et. al.*, 1980). The present report records a case of monocephalus diphygus monster in a water buffalo (Bubalus bubalis).

History: A multigravida buffalo at full term pregnancy delivered a monster in the second parturition. Slight obstetrical assistance was required for expulsion of foetus, otherwise the parturition was normal and foetus was delivered in anterior presentation. No history of any monstrosity was reported either from the dam or sire used for other buffaloes.

Anatomical description: The monster was a well developed female foetus weighed 36 kg. It was alive for 20 hours. There was no evidence of gross tissue degeneration and mummufication. Skeletal ankylosis was not noticed. The calf had two pairs of hind limbs and one pair of forelimbs (Tetrapus dibrachius - Fig)

The thorasic cavity enclosed one heart, two lungs, one esophagus and an intact diaphragm. Bifurcation started from the 10th thorasic vertebra and continued by two separate sets of lumbar, sacral and coccygeal vertebrae. The liver, spleen, pancrease gall bladder and intestine upto the end of iluem appeared single. Due to vertebral deformity the topography of the liver was grossly abnormal with unusual impression on its viseral surface. Ileum divided at the terminal end feeding two sets of large intestine each comprising of caecum, colon and rectum. On the dorsal side of the body a pouch has been formed between the bifurcated segment which accomodated the ansaspirals. The gentio urinary tract was symetrically divided and each segment comprised of one kidney, one ovary, one oviduct and one hypoplastic uterine horn without discernible cervix and vagina. The Urinary bladder was present in both pelvis which was fed by corresponding kidney.

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