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Effect of Superovulatory Response on embryo Recovery and Their Quality in Crossbred Cattle

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ABSTRACT

Effect of superovulatory response on embryo recovery and their quality was studied in crossbred cattle. Percent donors yielding eggs and average number of embryos/ova recovered per donor increased with the increase in ovulation rate. Fertilization rate of eggs were 100, 52.94 and 37.50 percent in donors with 3 to 6, 7 to 10 and more than 10 CL groups respectively, indicating decline in fertilization rate with the increase in ovulation rate. Of the flushed donors 15.38 percent did not produce any egg while 69.23 and 15.38 percent donors yielded 1 to 5 and ≥ 6 eggs/embryo, respectively.

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Ovarian response following superovulatory treatment influences embryo production and their quality in cattle. Some investigators have reported increase in embryo recovery with the increase in ovulation rate (Menino and Wright, 1978; Olivera *et al.*, 1984; Foote *et al.*, 1989), however, Sreenan (1978) observed decrease in embryo recovery with the increase in ovulation rate. On the other hand Shea *et al.*, (1976) and Critser *et al.*, (1980) did not observe any effect of ovulation rate on embryo recovery. Deterioration in the quality of embryo with the increase in ovulation rate has also been reported (Agarwal *et al.*, 1995). In the present paper effect of superovulatory response on embryo recovery and their quality in crossbred cattle is reported.

MATERIALS AND METHODS

Sixteen crossbred cattle (Hariana crosses with Holstein-Friesian, Brown Swiss and Jersey) having no gross abnormalities of genital organs were selected from the institute's dairy herd. All animals were cyclic and maintained under standard feeding and managemental conditions. The animals were superovulated with a single intramuscular injection of 2000 IU PMSG (pregnant mares serum gonadotrophin, Folligon, Intervet International, Holland) during mid luteal phase (days 10-12) of the cycle followed by a single intramuscular injection of 25 mg PGF_2 alpha (Dinofertin, Alved Pharma and Food Ltd., India) 48 hrs later. All the animals were subjected to detection of estrus after the injection of PGF_2 alpha. The estrus was detected twice daily i.e. morning and evening with the help of a vasectomized teaser bull and visual observation of external estrus symptoms. Donors were inseminated with frozen semen at 0.12 and 24 hrs after the onset of estrus. The ovarian response was determined by rectal palpation of corpus luteum on the ovaries on the day of flushing. Embryos were collected by non-surgical method of uterine flushing on day 6 ro 7 post-estrus (day 0 = day of superovulatory estrus). Modified Dulbecco's phosphate buffer saline containing 0.2% bovine serum albumin fraction V was used as flushing medium. Two way foley catheter for uterine flushing and an embryo concentrator (Emcon, Vet. Concepts Inc., USA) were used.The screening and evaluation of embryos was done morphologically under stereozoom microscope. Embryos were classified according to criterion described by Shea (1981) as excellent, good, fair and poor.

Excellent, good and fair quality embryos were considered as transferable.

RESULTS AND DISCUSSION

Results pertaining to the effect of superovulatory response on embryo recovery and their quality in different classes of donors are given in Table 1. Donors, based on superovulatory response, were divided into five categories viz. 0, 1-2, 3-6, 7-10 and >10 CL. The percentage of donors in the above categories were 0.0, 6.25, 50.00, 31.25 and 12.50, respectively. The results indicated that majority of donors (81.25%) were in the category of 3-10 CL and only 12.5% donors had more than 10 ovulations. Donors responded to superovulation treatment ≥3 CL, 93.75%) were flushed non surgically. Percent donors yielding eggs and average number of embryos/ova recovered per donor increased with the increase in ovulation rate. Several workers have reported positive relationship between ovulation rate and number of embryos/ova recovered (Menino and Wright, 1978; Olivera *et al.,* 1984; Foote *et al.,* 1989) which is in agreement to the findings of the present study, however, Shea *et al.,*. (1976) and Critser *et al.,*. (1980) did not find any effect of superovulatory response on embryo recovery rate. The number of unfertilized eggs recovered per donor increasd with the increase in ovulation rate indicating decrease fertilization rate with the increase in ovulation rate. Similar observations were made by Church and Sea (1976) and Agarwal *et al.,*. (1995).

Data on embryo production revealed that 15.38% donors did not produce any egg and majority of donors (69.23%) yielded eggs between 1 and 5. However, only15.38% donors produced ≥6 eggs / embryo. Seidel and Seidel (1989) and Agarwal *et al.,*. (1995) have also reported that approximately one-third donor don't produce any embryo which is inagreement to the findings of the present study.

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Table 1. Effect of superovulatory response on embryo recovery and their quality in cattle.

Category of donors/ parameters studied	0 CL	1-2 CL	3-6 CL	7-10 CL	<10 CL
Animals superovulated (n=16,%)	0	1 (6.25)	8 (50.00)	5 (31.25)	2 (12.50)
Donors flushed	0	0	6	5	2
Donors yielding eggs (%)	0	0	4 (66.66)	5 (100.00)	2 (100.00)
Average number of embryos/ova racovered per donor	0	0	1.66±1.36	3.40±2.07	4.00±4.24
Fertilized eggs (%)	0	0	1.66±1.36 (100.00)	1.80±1.78 (52.94)	1.50±0.70 (37.50)
Transferable embryos (%)	0	0	0.33±0.51 (20.00)	1.00±1.41 (29.41)	0.50±0.70 (12.50)
Degenerated embryos (%)	0	0	1.33±1.21 (80.00)	0.80±1.09 (23.52)	1.00±0.00 (25.00)
Unfertilized eggs (%)	0	0	0	1.60±1.51 (47.05)	2.50±3.53 (62.50)

REFERENCES

Agarwal, S.K., Taneja, V.K., Yadav, M.C. and Shankar, U. 1995. Influence of ovulation rate on recovery and quality of embryos in crossbred cattle. Indian J. Dairy Sci.*48:* 72-74.

Church, R.B. and Shea, B. 1976. Some aspect of bovine embryo transfer. Anim. Breed. Abstr.*45:* 168.

Critser, J.K., Rowe, R.F., Delcampo, M.R. and Ginther, O.J. 1980. Emmbryo transfer in cattle : Factors affecting superovulatory response, number of transferable embryos and length of post treatment estrous cycle. Theriogenology,*13* 397.

Foote, R.H., Allen, S.E. and Henderson, B. 1989. Buserelin in a superovulatory regimen for Holstein cows : II yield and quality of embryos in commercial herds. Theriogenology, *31:* 385-392.

Menino, A.R. Jr. and Wright, R.W. Jr. 1978. The influence of ovulation number and ovarian dimension on embmryo production in superovulated cattle. Theriogenology, *9*:259.

Olivera - Angel, M., Voss, M.J. and Holtz, W. 1984. Recovery rate and quality of embryo collected from suckled cows and beef heifers after superovulation with PMSG. Theriogenology *22*:553-562.

Seidel, G.E.Jr. and Seidel, S.M. 1989. A training manual for embryo transfer in cattle. Food and Agricultural organization of the United Nations, Rome, 128, pp.

Shea, B.F. 1981. Evaluating the bovine embryo. Theriogenology.*15:*31-42.

Shea, B.f., Hines, D.J., Lightfoot, D.E., Ollis, G.W. and Olson, S.M. 1976. The transfer of bovine embryos. Anim. Breed. Abstr.*45:*755.

Sreenan, J.M. 1978. Non-surgical egg recovery and transfer in cattle. Vet. Rec. *109:*77-80.

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Superovulatory Response and Quality of Embryos In Ovine FSH and PMSG treated Tellicherry Goats[1]

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ABSTRACT

Twelve healthy, parous and cyclical Tellicherry goats were divided equally into two groups as FSH-O and PMSG group and treated for superovulation with ovine follicle stimulating hormone and pregnant mare serum gonadotrophin, respectively. The mean number of ovulation was significantly higher in FSH-O group (21.83 ± 1.99) than PMSG group (11.33 ± 2.67). The mean number of total, fertilized, transferable and non-transferable embryos were 16.16 ± 2.17, 14.33 ± 1.87, 13.16 ± 1.74 and 1.17 ± 0.40 in FSH-O group, which is significantly higher than the corresponding mean values 5.0 ± 1.37, 4.5 ± 1.31, 4.16 ± 1.14 and 0.33 ± 0.21 in PMSG group, respectively. The mean number of anovulatory follicles and lunfertilized ova showed no significant difference beween two groups.

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The type and amount of gonadotrophin used for superovulation are the major factors causing marked variability in superovulatory response (Armstrong *et al.*, 1983; Pendleton *et al.*, 1992). PMSG and FSH are the two commonly used superovulating agents in goats. FSG of porcine origin was reported to result in increased ovulation rate with low incidence of anovulatory follicles than PMSG (Nuti *et al.*, 1987). In recent days, FSH extracted from ovine pituitary is available in the market for superovulation. There is a paucity of information on the use of FSH of ovine origin in Indian goats for superovulation. Hence, the present investigation was undertaken in Tellicherry goats, a well known dual purpose South Indian breed with the objective to study the effect of ovine FSH and PMSG on the superovulatory response, embryo recovery and embryo quality.

MATERIALS AND METHODS

Twelve healthy, parous and cyclical Tellicherry goats weighing between 20 and 30 kgs were divided into two equal groups as FSH-O and PMSG group. The estrous cycle in all the goats was controlled by 3 mg norgestomet ear implant (Syncromate-B, Animals health Inc., USA) combined with 0.5 ml injection containing 1.25 mg estradiol valerate and 0.75 mg norgestomet at the time of implant insertion (Day O). The superovulation treatment was initiated on day 9 in FSG-O group with 180 mg NIH-FSH-S1 ovine follicle stimulating hormone (Ovagen, Immuno chemical products Ltd., New zealand) Subcutaneously at the dose rate of 22.5 mg NIH-FSH-S1 twice daily at 12 hours interval for 4 days. All the goats in PMSG group were treated with 1000 IU PMSG (Folligon, Intervet international, Holland)

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intramuscularly as a single dose on day 9. The ear implants were removed on day 11 and 10 mg PGF2a (Lutalyse, Upjohn, Belgium) was administered 12 hours before implant removal in all the goats of both the groups. All the does were bred to fertile bucks at 36,48 and 60 hours after implant removal. The embryo collection was performed surgically from the fallopian tubes 3 days after the first service using flushing media composed of Dulbecco's phosphate buffered saline solution added with fetal calf serum at 10% level. During embryo collection, both the right and left ovaries were observed for number of ovulations and anovulatory follicles. The oviductal flushings were screened under Nikon stereozoom microscope. The number of embryos and unfertilized oocytes were carefully counted and recorded and then the fertilized embryos were graded based on their morphological characteristics as excellent, good, fair and poor as described by Kathiresan (1993). The difference in number of corpora lutea, anovulatory follicles and quality of embryos between FSH-O and PMSG group was analysed statistically using student 't' test.

RESULTS AND DISCUSSION

Mean number of ovulations, anovulatory follicles, transferable and non-transferable embryos are presented in the Table. Statistical analysis revealed that the FSH-O group had significantly higher ($P>0.01$) number of ovulations than PMSG Group. Batt *et al.*,. (1993) observed similar findings in goats treated with Ovagen and PMSG. Higher number of ovulation with FSH than PMSG treatment was also reported by Armstrong *et al.*,. (1983) and Selgarth *et al.*,. (1990). This may reflect the greater prevention of follicles from entering atresia or reversal of atretic process in FSH treated goats. The mean number of ovulations observed in PMSG group was in agreement with the reports of Mahmood *et al.*,. (1991) and Pendleton *et al.*,. (1992). There were more number of abnormal, prematurely regressing corpora lutea in PMSG group. This is in agreement with the observations of Pendleton *et al.*,. (1992). The number of anovulatory follicles showed no significant difference between two treatments. However, numerically it was higher in PMSG group than FSH-O group. This might be due to continued recruitment of follicles caused by persisting high circulating level of PMSG as a result of its long half life.

The total embryo recovery was 16.16 ±2.16 in FSH-O group and 5.0 ± 1.37 in PMSG group. It was significantly higher ($P < 0.01$) in FSH-O group than PMSG group. Similar to this report, several investigators reported higher embryo recovery in FSH than PMSG treated goats (Armstrong *et al.*, 1983; Mahmood *et al.*, 1991; Pendleton *et al.*, 1992). The low embryo recovery in PMSG group might be due to excessive estradiol level in the circulation during early luteal phase (McIntosh *et al.*, 1975) which resulted in premature luteal regression and abnormalities in embryo transport (Armstrong *et al.*, 1983).

FSH-O treatment resulted in 54(62.8%), 25(29.1%), 3(3.5%) and 4(4.6%) excellent, good, fair and poor quality embryos, respectively. Where as in PMSG treatment, out of 27 embryos recovered, 16(59.3%) were excellent, 9(33.33%) were good and 1(3.7%) each in fair and poor quality embryos. The mean number of transferable and non-transferable embryos were 13.16 ± 1.74 and 1.17 ± 0.40 in FSH-O group, which is significantly higher than corresponding values 4.16 ± 1.14 and 0.33 ± 0.21 in PMSG group, respectively. The major factors responsible for the low yield of transferable embryos in PMSG group were the presence of

prematurely regressing corpus luteum and earlier onset of estrus in PMSG treated goats (Tervit *et al.,* 1983).

Almost all the recovered embryos in the present study were between 2 to 8 cell stage. This is in agreement with the findings of Agrawal *et al.,*. (1982) and Mahmood *et al.,*. (1991). The variability in the developmental stages might be due to occurrence of ovulation over a period of 12 hours in superovulated goats (Baril and vallet, 1990).

Table: Mean Suprovulatory Response and Embryo quality in Tellicherry Goats

S.No	Parameters	FSH-0 group	PMSG Group
1.	No. of ovulations	21.83±1.99[a]	11.33±2.67[b]
2.	No. of Anovulatory	8.66±1.75	10.00±0.81
3.	Total no. of embryos recovered	16.16±2.17[a]	5.0±1.37[b]
4.	No. of Fertilized embryos	14.33±1.87[b]	4.5±1.31[b]
5.	No. of Transferable	13.16±1.74[a]	14.16±1.14[b]
6.	No. of Non-transferable	1.17±0.40[a]	0.33±0.21[b]
7.	No. of unfertilized ova	1.83±0.87	0.5±0.22

means bearing different superscript between columns differ significantly (P>0.01.

REFERENCES

Agrawal, K.P., IV Mongha and N.K. Bhattacharyya (1982). Collection and Transfer of Embryos in goats : Surgical Method, Indian vet. J., *59:* 298-303.

Armstrong, D.T., A.P..Pfitzner and G.M. Warnes and R.F. Seamark (1983). Superovulation treatments and embryo transfer in Angora goats, J. Reprod.Fert., 67: 403-410.

Baril, B. and J.C. Vallet (1990). Time of ovulations in dairy goats induced to superovulate with Porcine Follicle Stimulating Hormone during and out of the breeding season. Theriogenology, *34:* 303-311.

Batt, P.A., I.D. Killeen and W.N. Cameron (1993). Use of single or multiple injections of FSH in embryo collection programmes in goats. Reprod. Fert. Dev., *5* 49-56.

Kathiresan D. (1993). Studies on superovulatgory response in prepubertal goats. Thesis submitted to the Tamil Nadu Veterinary and Animal Sciences University, Madras for the degree of Doctor of Philosophy.

Mahmood, IS., G.L. Koul and J.C. Biswas I(1991). Comparative efficacy of FSH-P and PMSG on superovulation in Pashminagoats. Theriogenology, *35:* 1191-1196.

Nuti, L.C., B.S. Minhas, W.C. Baker, J.s. Capshart and P. Marrack (19987). Superovulation and recovery of zygotes from Nubian and Alpine dairy goats. Theriogenology, *28:* 418-488.

Pendleton, IR.J., C.R. Younga, R.W. Rorie, S.H. Pool, M.A. Memon and R.K. Godke (1992). Follicle Stimulating Hormone Versus Pregnant Mare Serum Gonadotrophin for Superovulation of dairy goats. Small Ruminant Res., *8:* 217-224.

Selgarth, J.P., M.A., Memon, T.E. Smith and K.M. Ebert (1990). Collection and transfer of micro injectable embryos from dairy goats. Theriogenology, *34:*1195-1205.

Tervit, H.R., P.G. Goold, K.D. McKenzie and D.K. Clarkson (1983). Techniques and success of embryo transfers in Angora goats. New Zealand Vet.J., *31:* 67-70.

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Response to PMSG Priming on superovulations and Embryo Recovery in Bharat Merino Sheep

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ABSTRACT

The effect of PMSG priming in early cycle prior to superovulation treatment was examined in Bharat Merino ewes. Ewes (16 no.) were allocated in two groups. Estrus was synchronised with 2 doses (10 mg) of prostaglandin F2 alpha (PGF) at 10 days interval. Group 1 ewes received a single injection of PMSG (400 IU) in conjuction with FSH (Ovagen, 7.4 mg) over 4 days starting from day 7 of first PGF injection. In the group 2, superovulation treatment was same as in group 1 except cycle (day 4 after first PGF injection) and other dose of 200 IU as in group 1. All the ewes in group 2 and 75% in group 1 exhibited heat after second PGF injection. Early cycle PMSG administration did not influence the subsequent ovulation rate, ovarian response, egg recovery and fertilization. Proportion of ewes responded to treatment (>2CL) was higher in PMSG primed ewes (100 vs 50%, $P<0.05$).

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Superovulation is an important event in multiple ovulation and embryo transfer programme for shedding more than normal number of oocytes from ovaries. Individual variability to the response to exogenous gonadotrophin is one of the major limitation in successful embryo transfer (Hahn, 1992). Recent research efforts are directed to improve the superovulatory effeciency and shedding of quality oocytes. Superovulation has been attempted by using a regimen which combines both FSH and PMSG (Ryan *et al,* 1991), administring a priming dose of FSH early in estrus cycle or prior to usual FSH treatment in late luteal phase (Ware *et al*, 1988) and use of homologous gonadotrophin (FSH from sheep pituitaries - Dingwall *et al*, 1993). This study was undertaken to examine the efficacy of two regimen of superovulation treatments consisting of PMSG and FSH (Ovagen) in Bharat Merino ewes and to investigate the response of a priming dose of PMSG in early estrus cycle.

MATERIALS AND METHODS

Bharat Merino ewes (n=16; 32-38 kg body weight) of known fertility, were randomly allocated in equal number in two groups and maintained under standard farm practices. Bharat Merino is a elite fine wool strain developed at the institute through crossing native sheep with Rambouillet rams and selection over the years. The experiment was performed during August when major breeding activities commence.

The onset of estrus in ewes was synchronised by treatment with two injection of prostaglandin F2 alpha (Lutalyse, Unichem India), 10.0 mg each at an interval of 10 days. Superovualtion in group 1 was induced by a single intramuscular injection of 400 IU PMSG (Folligon, Intervet, Netherlands) in conjunction with 7.4 mg of FSH (Ovagen, ICP New Zealand) commencing at the time of PMSG treatment (on day 7 of first PGF injection) over 4 days @ two injection per day (0800 and 1700 hrs). In group 2, treatment was similar to group 1 except PMSG dose which was divided in two equal amounts; one (200 IU) administered in early cycle (on day 4 after first PGF) and other (200 IU) as in

group 1. Ewes in estrus were deducted twice daily (morning and evening) by parading aproned ram and mated 2-4 times to a ram of proven fertility while in estrus. On day 3-6 post-mating laparoscopy was performed as per procedure described elsewhere (Naqvi *et al*, 1995) and ovulation points were counted. Ewes having >2 ovulation (estimated by counting corpora haemorrhgica, CL) were referred as responder and subjected to laparotomy. Ovaries were inspected and number of CL and large unovulated follicles (LF, <5 mm in diameter) were counted. The ovarian response was estimated by summing up number of CL and LF Each horn of uterus and oviducts were flushed retrogradely as per modified procedure described by Hunter *et al*, (1955) with 20 ml of phosphate buffer saline (PBS) supplemented with sodium pyruvate (0.036 g/1), antibiotics (Streptomycin sulphate 0.05 g/1; Penicillin-G sodium 1 million units) and 3% (w/v; bovine serum albumin). Recovered embryos were counted and examined for quality under stereozoom microscope. Data pertaining to proportions were analysed using Chi-square analysis and means were compared using student "t" test.

RESULTS AND DISCUSSION

Data on estrus synchronisation, superovulation and embryo recovery are presentd in table. All the ewes in group 2, which received a priming dose of PMSG, and 75% (6/8) in group 1 exhibited heat within 48 hours of second PGF treatment. Interval between PGF injection and onset of heat (28.5±2.01 vs 26.9±2.07 hrs.), and duration of heat (45.5±5.97 vs 43.1±6.49 hrs.) were comparable in the two groups and did not approach to statistical significance ($P<0.05$). The mean ovulation rate was (9.3 vs 9.3; $p<0.05$) and ovarian response (10.6 vs 11.3; $p<0.05$) in ewes that exhibited heat in group 1 and 2, respectively. A higher proportion of ewes responded to superovulation treatment in group 1 than in group 2 (8/8 vs 4/8; $p<0.05$). The egg recovery rate, fertilization rate and number of transferable embryos were not influenced due to PMSG priming in ewes which responded to superovulation treatment.

From the results, it is evident that a large proportion of ewes did not respond to treatment of superovulation without PMSG priming. However, no beneficial effect was observed due to PMSG prming in increasing the superovulatory effeciency and embryo recovery. Ware *et al* (1988) reported that early cycle FSH priming did not increase the subsequent ovulation in sheep. Gonadotrophin priming treatment in cows increased (Rajamahendran *et al*, 1987), decreased (Lussier and Carruthers, 1989), or had to effect (Grey *et al*, 1992) on superovulation. Total number of transferable embryos per ewe treated, among the PMSG primed ewes in this study, was in close agreement with similar priming treatment (Ware *et al*, 1988). The failure of PMSG priming to increase ovulation rate in this study may be related to lesser time interval (4 days) between priming and superovulatory treatments. Considerably larger period (about 2 estrous cycles) is required for growth of preantral follicle to preovulatory stage (Armstrong, 1993). Recent ultrasonographic study of sheep ovaries during estrus cycle has demonstrated that ovarian antral follicle growth and regression occurs on many days of the estrus cycle (Ravindra *et al*, 1994). Gonadotrophin priming treatment might have increased the sensitivity of ovaries to subsequent superovulatory treatment and thereby increased the number of ewes ovulating.

Table: Effect of PMSG priming on superovulation and embryo recovery in Bharat Merino sheep.

		Without PMSG priming (Gr-1)	With PMSG priming (Gr-2)	Statistical Significance
1.	No. of ewes treated	8	8	
2.	No (%) of ewes in estrus	6(70)	8(100)	NS
3.	No. of CL/ewe exhibited estrus	9.3±3.41	9.3±1.06	NS*
4.	No. of large follicles	2.0±0.68	1.4±0.53	NS
5.	Ovarian response/ewe	11.3±1.59	10.6±0.5	NS
6.	No (%) of ewes responded (<2CL) to treatment.	4(50)	8(100)	*
7.	No. of ewes flushed	4	8	
8.	No (%) of ewes yielded eggs	4(100)	7(75)	NS
9.	Overall egg recovery rate (%)	37.5	43.2	NS
10.	Percent of fertilized eggs	33.3	37.5	NS
11	No.of eggs recovered/ewe flushed	5.3±1.9	4.0±0.8	NS
12.	No.of fertilized eggs recovered/ewe flushed	1.8±1.44	1.5±0.87	NS

NS = Non - significant at level 5%, *=P>0.05

REFERENCES

Armstrong, D.T. (1993). Recent advances in superovulation in cattle. Theriogenology.*39:* 7-24.

Dingwall, W.S., Femine, K., Fitzsimos, J. and Mckelvey, W.A.C. (1993). MOET in suffolks. Increasing the superovulation rate. Animal Production *56:* 444-48.

Gray, B.W., Cartee, R.E., Stringfellow, D.A., Riddell, M.G., Riddell, K.P. and Wright, J.C. (1992). The efforts of FSH-priming and dominant follicular regression on the superovulatory response of cattle. Theriogenology *37:* 631-639.

Hahn, J. (1992). Attempts to explain and reduce variablility of superovulation. Theriogenology *29:* 125-142.

Lussier, J.G. and Carruthers, T.D. (1989). Endocrine and superovulatory responses in heifers pretreated with FSH or bovine follicular fluid. Theriogenology *31:* 779-794.

Naqvi, S.K.K. and Gulyani, R. (1995). A quick method of embmryo transplantation in sheep. Indian J. Small Rum. *1:* (2) 22-24.

Rajamahendran, R., CAnseco, R.S. and Denbow, C.J. (1987). Effect of low dose of FSH given at the beginning of the estrus cycle and subsequent superovulatory response in Holsstein cows. Theriogenology *28:* 59-65.

Ravindra, J.P., Rawling, N.C., Evans, A.C.O. and Adams, G.P. (1994). Ultrasonographic study of ovarian follicular dynamics in ewes during the estrus cycle. J. Reprod. Feroil. *101:* 501-509.

Ryan, J.P., Hunton, J.R. and Maxwell, W.M.C. (1991). Increased production of sheep embryos following superovulation of Merino ewes with a combination of PMSG and FSH. Reprod. Fertil. Dev. *3:* 551-568.

Ware, C.B., Northey D.L., Boland, M.P. and First, N.L. (1988). Early cycle FSH-P priming as a prelude to superovulating gonadotrophin administration in ewes and heifers. Anim. Reprod. Sci. *16:* 97-105.

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Effect of Buserelin on Superovulatory Response in Crossbred Cattle Treated with PMSG and Folltropin V

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ABSTRACT

In the present study the effect of Buserelin on superovulatory response in crossbred cattle was studied. A nonsignificant ($P>0.05$) increase in the ovulation rate, egg recovery and mean transferable embryos and a significant ($P<0.05$) decrease in the number of unovulated large follicles was observed in PMSG + GnRH treated sub-group than PMSG alone.

A significantly higher ($P< 0.05$) ovulation rate, egg recovery and transferable embryos and a significantly lower ($P<0.05$) number of unovulated large follicles were observed in Folltropin + GnRH treated sub-group than Folltropin alone. Folltropin was better than PMSG for superovulation but PMSG + GnRH yielded similar results as that of Folltropin alone.

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Previous experiences have indicated a better superovulatory response by FSH as compared to PMSG (Elsden *et al.,* 1978; Monniaux *et al.,* 1983) but still PMSG may prove to be suitable (atleast in developing countries) due to its lower cost, single dose schedule and easy availability. An uncontrolled superovulation with PMSG may be associated with its relatively long circulating half life (Moor *et al.,* 1985) Murphy *et al.,* 1991) which results in excessive follicular development and failure of ovulation. Reports indicate that GnRH or it analogue promote LH surge of higher intensity (Phillippo and Rowson, 1975; Schams *et al.,* 1978) which may augment the ovulatgory process.

The present study was, therefore, designed to investigate whether Buserelin (GnRH analogue) can improve the superovulatory response, embryo recovery and quality parameters when used with PMSG and FSH.

MATERIALS AND METHODS

A total of twenty nine crossbred cows (age 5-11 years) having normal estrous cycle and genital tract were selected for this study. They were atleast two months post-partum with a body weight ranging from 360-480 kgs.

In the first experiment, the animals (n=15) were superovulated with a single intramuscular injection 1500 IU) of PMSG (Folligon, Intervet Holland) on day 10 or 11 of the estrous cycle (day '0' = estrus). Estrus was induced by 3.75 mg Luprostiol (Prosolvin, Infar India Ltd), administered by intravulvo-submucosal route (ipsilateral to the side of CL) 48 hours after the gonadotrophin injection (Khanna *et al.,* 1995). The animals were divided into two sub-groups out of which sub-group I (n=8) served as Control while sub-group II cows (n=7) were treated with 8μg Buserelin(I/M injection) (Receptal, Hoechst India Ltd) with the first insemination.

In the second experiment, the animals (n=14) were superovulated with 400mg of Folltropin V (NIH-FSH-PI Std; Vetpharma Inc., Canada) in a four days tapering dose schedule (80/80, 60/60, 40/40, 20/20). The gonadotrophin was administered intramuscularly at 12 hours interval between days 9 to 12 and the estrus was induced 48 hours after gonadotrophin initiation (as in previous experiment). Out of these, sub-group I animals (n=7) served as control whereas sub-group II cows(n=7) were treated with Buserelin (as in sub-group II

of experiment 1). Estrus was detected twice daily by teaser parading and visual signs of estrus. The animals were inseminated with liquid semen (sperm conc. 20 million progessively motile supermatozoa per ml) 8 hours after the standing estrus and repeated twice at an interval of 12 hours. The ovarian response was estimated in terms of number of corpus luteum and unovulated large follicles (10 mm dia) on day 7, per rectally. The non-surgical uterine flushing was done on day 7 by gravitational method using two way foley's catheter and the embryo quality was assessed under stereozoom microscope (Lindner and Wright, 1983).

Statistical analysis was done by Micro-32 computer and means were compared using 't' test (Snedecor and Cochran, 1968).

RESULTS AND DISCUSSION

In experiment 1 higher ovulation rate was observed in PMSG + Buserelin alone (7.00±1.30) as compared to PMSG alone (5.57±0.75) (Table 1). The difference was statistically nonsignificant (P>0.05). There was a significant reduction (P<0.01) in the number of unovulated large follicles (≥ 10 mm dia) in Buserelin treated group (2.57±0.36) than in PMSG alone (5.00±0.31). Embryo recovery percentage (67.3 Vs. 53.8), average number of embryos + ova recovered per donor and the average number of transferable embryos were higher in PMSG + Buserelin group than in PMSG alone. The differences were, however, nonsignificant (P>0.05). Our study is in agreement with the other studies indicating enhanced ovulation rate and lower number of unovulated follicles (Newcomb, 1980; Guay and Bedoya, 1981; Takahashi and Kangawa, 1984). Foote *et al.,* 1989) and a higher number of transferable embryos (Savage and Mapleloft, 1984; Foote *et al.,* 1989) in PMSG + GnRH treated group.

In the experiment II results indicated a significantly higher(P<0.05) ovulation rate (12.71±2.84 vs. 7.60±1.46), average number of embryos + ova recovered(10.57±3.02 vs. 3.8±1.31) and average number of transferable embryos (3.43±0.92 vs. 0.90±0.37) in Folltropin + Buserelin than Folltropin alone treated group. The average number of unovulated follicles were significantly lower (P<0.05) in Folltropin + Buserelin than Folltropin alone treated group (0.43±0.20 vs. 0.80±0.37). These findings were similar to those reported earlier in the literature (Savage and Mapleloft, 1984; Laurinick *et al.,* 1993). A higher ovulation rate in Folltropin + Buserelin treated group may be due to controlled ovulation by a timely LH surge (Savage and Mapletoft, 1984).

Table: Superovulatory response in crossbred cows treated with PMSG, PMSG + Buserelin, Folltropin and Folltropin + Buserelin

Superovulation Treatment	Experiment I		Experiment II	
	PMSG	PMSG + Buserelin	Folltropin	Folltropin + Buserelin
Animals (n)	8	7	7	7
Animals responding to super-ovulation (3 CL) (%)	7(87.5%)	7(100%)	5 (71.4%)	7 (100%)
Ovulation rate	5.57±0.75[a]	7.00±1.30[1]	7.60±1.46[a]	12.71±2.84[b]
Average number of ova/ embryo recovered	3.00±0.90[a]	4.71±1.42[a]	3.80±1.31[a]	10.57±3.02[b]
Average number of transferable embryos (%)	1.00+0.30[a] (33.33)	1.57±0.42[a] (33.33)	0.90+0.37[a] (23.68)	3.43±0.92[b] (32.45)

Average number of degenerated embryos (%)	0.72±0.10 (24.00)	1.14±0.28 (24.20)	1.10±0.80 (28.95)	5.71±1.21 (54.02)
Average number of unfertilised ova (%)	1.28±0.56 (42.67)	2.00±1.69 (42.47)	1.8±0.73 (47.37)	1.43±0.65 (13.53)

Mean values bearing different superscript differ significantly (P<0.05).

REFERENCES

Elsden, R.P., Nelson, L.D. and Seidel, G.E. Jr. (1978). Superovulating cows with FGH and PMSG. Theriogenol., *9:* 17-26.

Foote, R.H., Alen, S.E. and Henderson, B. (1989). Buserelin in superovulatory regimen for Holstein cows II. Yield and quality of embryos in commercial herd. Theriogenol., *31:* 385-392.

Guay, P. and Bedoya, M. (1981). A study of equivalence between rectal palpation, laproscopy, laparotomy and ovarian dimension for evaluation of the ovarian response of PMSG superovulated cows. Can. Vet. J. *22:* 353-355.

Khanna, S., Ansari, M.R., Majumdaer, A.C., Taneja, V.K. and Mohanty, T.K. (1985). Estrus induction in crossbred cattle using low dose of Luprostiol. Indian J. Anim. Sci., *65:* 193-194.

Laurinick, J., Hyttel, P., Gratenau, P. and Greve, T. (1993). Character of preovulatgory follicles and oocytges after different superovulatory treatments in heifers. Theriogenol., *39:* 545-551.

Lindner, sG.M. and Wright, R.J. Jr. (1983). Bovine embryos, morphology and evaluation. Theriogenol., *2:* 407-416.

Monniaux, D.,Chupin, D., Saumande, J. (1983). Superovulatory responses in cattle. Theriogenol., *19:* 55-81.

Moor, R.M., Osborn, J.C. and Crossby, I.M. (1985). Gonadotrophin induced abnormalities in sheep oocytes after ovulation. J. Reprod. Fertil. *74:* 167-172.

Newcomb, R. (1980). Investigation of factors affecting superovulation non-surggical embryo recovery from lactating British Friesian cows. Vet. Rec. *109:* 48-54.

Phillippo, M. and Rowson, L.E.A. (2975). Prostaglandin and superovulation in the bovine. Ann. Biol. Anim. Biochem. Biophys. *15:* 233-240.

Savage, N.C. and Mapletoft, R.J. (1984).Superovulation in cow using estradiol 17B or GnRH in FSH-p regimen. Theriogenol. *21:* 259 (Abst).

Schams, D., Menzer, G.H., Schallenberger, NB., Hoffman,B. andHaha, R. (1978). Some studies on PMSG and an endocrine response aftger application for superovulation in catgtle. In: Control of Reproduction in Cow. Ed. Sreenan, J., CEL, Galway, Luxemburg, pp.122.

Snedecor, G.W. and Cochran, W.G (1968). Statistical Methods. 6th edn. Oxford and IBHPublishing Co., 66, New Delhi, India.

Takahashi, Y. and Kangawa, W. (1984). Effect of LH-RH analogue on the ovulation rate and embryo quality in heifers superovulated with PMSG and PGF_2x. Jap. J. Vet. Res. *32:* 183.

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Oestrous Synchronization and Superovulation by PGF_2 alpha in the Presence of different Level of PMSG in Malpura ewes

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ABSTRACT

Comparative study of hormonal synchronization by PGF_2 alpha 7.5 mg with different dose level of PMSG viz 1500 iu, 2000 iu and 2500 iu were studied. 50 per cent ewes exhibited oestrus with 44.67±0.15 hour's duration and 22.67±0.84 length of oestrus. An average superovulating response on both the overies observed in terms of CL and GF was 4.0 to 5.0 and 5.0 to 11.0 respectively. It is conlcuded that higher doses of PMSG increased more development of G.F. on overies.

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Embryo transfer has been extensively used as a research tool in recent years but its application in sheep breeding has not been substantial. The induction of superovulation and the yield of maximum numbers of embryos with good qualities are the most important factors for the success of embryo transfer procedure. For synchronization exogenous hormones such as progesterone and prostagladin are used to control oestrous.

In present investigation, PGF2 alpha (Prostaglandin F_2 alpha) are used in the presence of different level of PMSG to obtain the oestrus synchronization and super ovulation.

MATERIALS AND METHODS

Twelve cyclic mature healthy ewes approximately of same age group were selected from the sheep and Goat Research Centre, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola for the study. Housing and Managemental practices were identical. These ewes were divided into four groups and treated as follows. Group I. Inj. Dinofertin (PGF_2 alpha) 7.5 mg per ewe on day 0 and 13. Inj. Folligon (PMSG) 1500 iu on day 11. Group II. Inj. Dinofertin 7.5 mg per ewe on day 0 to 13, and inj. Folligon 2000 iu on day 11. Group III. Inj. Dinofertin 7.5 mg per ewe on day 0 and 13 and Inj. Folligon 2500 iu on day 11. Group IV, Inj. Dinofertin 7.5 mg per ewe on day 0 and 13. Oestrous detection observed at the end of treatment by aproned ram. Synchronization of oestrus in ewes were studied on the basis of (a) Time required for onset of oestrous after last hormonal injection (b) Duration of oestrous in hours from first mount to last mount of the aproned ram. For the study of superovulatory response (number of CL and GF) laprotomy operation at mid-ventral region of abdomen were performed aseptically, under local infiltration anaesthesia.

RESULTS AND DISCUSSION

Results indicate that average occurance of synchronised oestrus after the last hormonal injection in all groups was 50 per cent. Oela (1982) observed 60 per cent of female came into heat within 24 to 120 hours by 10 mg PGF_2 alpha and 800 iu PMSG. In present study, it is seen that 50 per cent ewes came into heat by using 7.5 mg PGF_2 alpha with increasing level of PMSG from 1500 iu to 2500 iu within 24 to 60 hours (average 44.67±0.15 hrs) of duration of oestrus with the length of oestrus from 20 to 24 hours

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(average 22.67±0.84). It could be concluded that duration and length of oestrus can be reduced by increasing the dose level of PMSG. These observations were correlated with Matose *et al*. (1987). However, Martinez *et al*. (1987) observed more time (84±33 hours) required for oestrus synchronization.

Mutiga and Baker (1982) superovulated Merino ewes using 1000, 1500 and 2000 iu PMSG on day 9 of oestrus cycle and 125 ug PGF_2 alpha 2 days before observed more CL and less unovulated folicles than present investigation. This could be attributed to climatic condition and variation of the breed. However, ovulation rate observed (4.0 to 0.5.0 CL and 5.0 to 11.0 GF) in the present study was more than Bankov *et al*. (1983) who observed oestrus synchronization with PGF_2 alpha and PMSG (750 iu and 100 iu) by ovulation rate averaged 3.4 to 6.1 per ewe.

An overall result in present study indicates that, higher doses of PMSG increased more development of graffian follicles on overies.

REFERENCES

Bankov, N.; Karoivanov, K.H.; Vlakhov, K.; Petroy, M.; Dobrevs, D. and Petrov, P. (1983). The possibility of inducing superovulation and obtaining embryos for transfer in sheep. Veterinar-home ditsinski Neiuki, *20(2):* 6-12. Anim. Breed. Abstr. (1984) 52(8): 4676.

Cela, M. (1982) Oestrous synchronization and superovulation in sheep by means of natural PGF_2 alpha and PMSG Anim. Breed. Abstr. *(1984) 52(1-3)²:* 584.

Martinez, N.; Rondon, Z.; Lopez, S.; Combellon, J.; Mendoja, R.; Perozo, D. and Arvelo, C. (1987) Use of prostaglandin (PGF_2 alpha) and Progestogen in the synchronization of oestrus in West African ewes. Anim. Breed. Abstr. (1977) *56(5) :* 2735.

Matos, M.; Martinez, N. and Arvelo, C. (1987) Oestrus synchronization by means of PGF_2 in West African ewes subjected to a different frequency and number of matings - Anim. BRead. Abstr. (1988)*56(5):* 2736.

Mutiga, E.R. and Baker, A.A. (1982) Superovulation response in Merino ewes to their PMSG dose levels. Theriogenology *17(1):* 100.

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Seroprevalence of infections in infertile cattle

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ABSTRACT

Sera from repeat breeder (n=75) and pregnant cattle (n=15) were collected from organized dairy herds and screened for brucellosis, listeriosis and leptospirosis. For brucellosis and listeriosis, 37.3% and 34.6% repeat breeder animals respectively were found positive as compared with none in pregnant animals. For leptospirosis, 17.3% repeat breeder animals were found positive as compared with 6.67% in pregnant animals. Therefore, these infections may be involved in causing repeat breeding problem.

Repeat breeding is one of the most important gynaecological problem in bovine. The condition may occur due to genetic, nutritional, hormonal and infections reasons. Among the infectious causes brucellosis, listeriosis and leptospirosis are not only important from reproduction stand point but they are also potentially hazarduous to human health since these diseases are transmissible from animal to man and vice-versa (Sane *et al.*, 1994). The association of brucellosis (Nagal *et al.*, 1991), listeriosis (Seeliger, 1961) and leptospirosis (Ellis, 1984) with abortion in cattle is well documented.

These infections are known to cause damage to reproductive tract particularly uterine endometrium which may led to repeat breeding problem. Therefore, the present study was carried out to find out the extent of involvement of these infections in repeat breeding condition.

MATERIALS AND METHODS

Serum samples from 75 repeat breeder cattle and 15 pregnant (conceived in ≥ 3 inseminations) animals were collected from organized dairy herds belonging to Bareilly district (U.P.). Sera were subjected to screening for the infections viz. brucellosis, listeriosis and leptospirosis. Brucellosis was detected by using sonicated antigen of *Brucella abortus* strain - 99 using an indirect ELISA (Batra *et al.*, 1989). Listeriosis was detected by using culture filterate antigen of *Listeria monocytogenes* MTCC 1143 employing an indirect - ELISA (Lhopital *et al.*, 1993). Leptospirosis was detected employing microscopic agglutination test (MAT) using 9 leptospiral strains (Cole *et al.*, 1973). These strains were *Leptospira interrogans serovars icterohaemorrhagiae, canicola, pomona, australis, autumnalis, ballum, hardjo, javanica and Leptospira hiffexa serovar patoc.*

RESULTS AND DISCUSSION

A total of 28 (37.3%) repeat breeder cattle were found positive (≥ 1:200 titre) for *Brucella abortus* infection where as none of the pregnant animal was positive. The difference between the groups was highly significant ($P<0.01$). As the titre increased, number of positive animals decreased. With the higher titre of 1:400, 1:800, 1:1600 and 1:3200; the positive repeat breeders were 16 (21.3%), 9 (12%), 2 (2.67%)

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and none, respectively. Studies on its prevalence by indirect-ELISA among aborted and incontact animal revealed that about 53.42% of aborted and 27.74% of incontact animals were positive for brucellosis (Chand *et al.,* 1990). Decrease in percentage of seropositive animals with increase in titre was also noticed by these workers, *Brucella abortus* seropositive cattle required more number of inseminations to became pregnant as compared to seronegative animals (Halder and Sen, 1990). This may be due to the presence of pathological lesions in non-pregnant uterus of seropositive animals (Chakraborty and Kwatra, 1980). Seropositive animals in present study may had such lesions which might have led to embryonic death and repeat breeding.

A total of 26 (34.7%) repeat breeder cattle were found seropositive (≥1:200 titre) to listeriosis compared with none in pregnant control group. The difference was highly significant (P>0.01). A decrease in number of seropositive repeat breeders was noticed with the increase in the titre of serum samples. The repeat breeders which turned out to be seropositive were 19 (25.3%), 13 (17.3%), 6(8%), 2(2.67%) and none at the titre of 1.400, 1:800, 1:1600, 1:3200 and 1:6400 respectively. Association of bovine infertility with seropositivity of *Listeria monocytogenes along with isolation of pathogen has been earlier reported (Perkucin et al.,* 1970; Srivastava et al. 1985). They have also reported variable agglutination titre. Seropositivity of 33.3% to listeriosis has been observed in repeat breeders by dot-ELISA (Sharma *et al.,* 1996).

Out of 75 repeat breeder cattle, 13 (17.3%) were found positive to leptospirosis (1:100 titre) as compared to only one (6.67%) in pregnant animals. The difference was statistically significant (P<0.05). Among the 13 positive repeat breeder cattle, 8 were positive for *L. biflexa* serovar patoc; 2 for *L. interrogans* serovar *ballum* and 2 for serovar *canicola* (Single serovar infection). One animal was positive for two strains viz. *L. interrogans serovar canicola* and *L. biflexa* serovar patoc. The pregnant animal was positive for *L. biflexa* serovar patoc. Earlier study on seroprevalence of leptospiral antibodies indicated that 7 out 51 (13.7%) sera from repeat breeder cattle were positive (Venugopal *et al.,* 1986). Lower prevalence in this report may be due to the use of only six strains as compared with nine in present study or may be because of variable prevalence of leptospirosis among different regions. In present study *L. biflexa* serovar *patoc* dominated over other serovars. Srivastava et al. (1990) have also reported the predominance of this serovar in U.P. Association of *L. interrogans* serovar *canicola* with repeat breeding in cattle was also documented by Kulasekar *et al.* (1990) but no association of *L. interrogans* serovar ballum with repeat breeding condition is available in the literature.

The results are suggestive of involvement of brucellosis, listeriosis and leptospirosis in repeat breeding conditions. Further extensive studies are needed to pin point pathophysiology of these infections in causing repeat breeding problem.

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REFERENCES

Barta, H.V., Chand, P., Mukharjee, L.G.R. and Sadana, J.R. (1989). Dot enzyme linked immunosorbent assay for detection of antibodies in bovine brucellosis Res. Vet Sci. *46 :* 143-46.

Chakraborty A, and Kwatra, M.S. (1980). Serological study of brucellosis and its histopathology in reproductive organs of non-pregnant slaughtered cows. Ind. J. Vet. Path Microb. *23 :* 125-28.

Chand, P., Sadana, J.R. and Batra, H.V. (1990). Comparision of a dot-ELISA and a plate-ELISA for bovine brucellosis diagnosis. Vet. Rec. *127 :* 169-70.

Cole, J.R., Sulzer, C.R. and Pursell, A.R. (1973). Improve microtechnique for leptospiral microscopic agglutination test. Applied Microb. *25:* 976-80.

Ellis, W.A. (1984). Bovine leptospirosis in the tropics : prevalence, pathogenesis and control. Prev. Vet. Med. *2 :* 411.21.

Halder, S.K. and Sen, G.P. (1990). Fertility and reproductive performance of *Brucella* seropositive cows. Ind. Vet. J. *67:* 278-79.

Kulasekar, K., Quayam, S.A. and Ratnam, S.C. (1990). Leptospirosis in repeat breeding and anestrous cattle. Ind. J. Ani. Repord. *11:* 128-30.

Lhopital, S., Marly, J., Pardon, P. and Barche, P. (1993). Kinetics of antibody productiona against listeriolysin - O in sheep with listeriosis. J. Clin Microb. *31 :* 1537-40.

Nagal, K.B., Katock, R.C. Sambyal, D.S. and Sharma, M. (1991). Seroprevalence of brucellosis in livestock of kangra valley in Himachal Pradesh. Asian J. Dairy Res. *10 :* 15-18.

Perkucin, R., Jerkovic, V., Zakula, S. and Markovic, P. (1970). Sterility of cows caused by *Listeria monocytogenes. Vet. Glan. 24* 589-93.

Sane, C.R. and others (1994).A text book of reproduction in farm animals (theriogenology) IInd ed. Verghere publishing House, Bombay.

Seeliger H.P.R. (1961). "Listeriosis". New York, Hafner.

Sharma, N.C., Barbuddhe, S.B. and Dhable, R.B. (1996). Dot-ELISA technique for testing *Brucella* and *Listeria* infections in repeat breeder bovines. 83rd Ind. Sci. Cong. held at Patiyala Jan. 1996 pp. 14-15.

Srivastava, N.C., Gupta, S.K. and Uppal, P.K. (1985). *Listeria monocytogenes* infection in infertile cows. Ind. Vet. J.*57* 270-72.

Srivastava, S.K., Verma, R. and Herbola, P.C. (1990). Seroprevalence of leptospirosis in animal and man in India. Ind. J. Anim. Sci.*60* 1439-41.

Venugopal, K., Ratnam, S. Raghavan, N. and Nachimuthu (1986). Prevalence of leptospiral antibodies in aborting and repeat breeding animals. Cherion. *15* 80-83.

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Incidence of reproductive disorders in relation to lameness in cows and buffaloes*

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ABSTRACT

The present study was conducted to find out the effect of lameness on the reproductive pattern of cattle and buffaloes. Among different farms screened in the Punjab State, 58.8 per cent lame cattle were found to have prevalent reproductive disorders. Anoestrus (23.97%) and repeat breeding (16.9%) were main observations in these animals in addition to cystic overies, cervicitis, abortion and retention of placenta. Interdigital wound, laminitis and overgrown hooves did have a major influence on reproductive pattern of lame animals. Buffaloes had minimal effect on reproduction in relation to lameness.

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Lameness, which is a multifactorial problem, has been thought to affect the production and reproductive performance of cattle (Collick, 1994). It is not a disease but a clinical sign of an underlying malady and it can have serious welfare implications for the affected individual. Diet of the animal has been implicated to play a significant role in the pathopsysiology of lameness. Moreover, lame animals develop disinclination to move and have reduced feed intake culminating into nutritive deficiencies, thus resulting into reduced body weight, short lactation period, delay in oestrus and poor breeding performance (Eddy and Scott, 1980; Lucy *et al.*, 1986).

The present study was conducted to study the various reproductive disorders prevalent in relation to lameness in the dairy animals in the Punjab State.

MATERIALS AND METHODS

A total of 2218 animals (cattle=1959, buffaloes=257) were examined at various Govt. and private dairy farms in the Punjab State to find the incidence of lameness and any effect of lameness on reproductive activity. The per-rectal examination was conducted on the animals found to be lame to evaluate the functional status of the reproductive organs. Different reproductive abnormalities like lack of cyclicity, repeat breeding, abortion, cystic ovarian disease etc. were recorded in each individual lame animal to find out the incidence of prevalent reproductive disorders in them and any relation, between the two.

RESULTS AND DISCUSSION

Among the total animals screened, 158 cattle (8.10%) and 10 buffaloes (3.89%) were found to have mild to severe degree of lameness. A total of 56.6. per cent among the lame cattle were found to have some or the other reproductive ailment. Anestrus and repeat breeding prevalent in 23.9 and 16.9 percent animals, respectively, constituted the major factors affecting the successful breeding. Cystic ovaries, retention of fetal membranes and abortion were encountered in 5.03, 5.60 and 2.51 per cent lame cattle, respectively. Miscellaneous causes viz. cervicitis, pyometra were observed in 2.51 per cent

* Part of M.V.Sc. thesis submitted by the first author to P.A.U., Ludhiana

cattle. Delayed oestrus, increasing calving to conception rate and increased number of services per conception have been reported in relation to lameness in bovines (Dews, 1978; Collick *et al.,* 1989).

Among different foot abnormalities, interdigital wound (28.3%) overgrown hooves (20.1%) followed by cork screw hoof (10.6%), laminitis (9.4%), hoof crack (9.4%) and white line disease (6.9%) were the main foot afflictions encountered (Table). In the animals having interdigital wound, anoestrus (26.8%) and repeat breeding (17.7%) were the most frequent reproductive problems followed by cystic overies (5.03%), and retention of placenta (5.60%). Repeat breeding was the most common symptom (33.3%) observed in lame cattle affected with laminitis. Animals with overgrown hooves and hoof cracks also had common problems of anoestrus and repeat breeding in 25.0 and 26.6 per cent and 15.6 and 13.5 per cent animals, respectively. Likewise, 17.3 per cent of animals having corkscrew feet anoestrus while 23.5 per cent were repeat breeders. 5.8 per cent animals either had incidence of retention of placenta or abortion. White line disease and miscellaneous causes of lameness like tendon injury, coronet swelling, etc. were reflected through anoestrus in 27.2 and 25.0 per cent of animals.

The hoof lesions have been found to be highly stressful to the animal due to tissue damage and pain. Higher incidence of anoestrus and repeat breeding in the lame animals might be due to enhanced plasma cortisol and B-endorphins under the influence of stress and pain (Mobeg, 1991; Whittakar *et al.,* .1983). Both cortisol and B-endorphins are the potent suppressor of hypothalamic pituitary axis which results in blockage of release of gonadotrophins (Nanda *et al.,* 1989). It might thus, delay the onset of cyclicity and decrease the breeding efficiency of the animals. Also, the cow's ability to compete for food may be impaired by lameness, which could lead to reproductive failure in animals (Peelar *et al.,* 1994).

Buffaloes, on the other hand, did not have any significant influence on reproduction in relation to lameness. Out of lame buffaloes (10/257), four were pregnant; two were noncyclic while one buffalo was repeat breeder, thus indicating that lame buffaloes may continue to have nearly normal reproductive behaviour.

Looking into increasing incidence of lameness and its impact on animal reproduction and production, it thus, becomes imperative to monitor the hoof health and follow proper hygienic measures so as to further reduce the incidence of foot lesions and associated reproductive disorders.

Table 1. Incidence of various reproductive disorders at different conditions in relation to various foot disorders in cattle.

Type of Laseness	Total lame animals	Anoes-trus	Repeat Breeding	Cystic Ovar-ies	Retention of Placenta	Abor tion	Niscel-laneous	Total
Interdigital wound	45	12 (26.6)	8 (17.7)	4 (8.8)	2 (4.4)	1 (2.2)	1 (2.2)	28 (62.2)
Overgrown hooves	32	8 (25)	5 (15.6)	1 (3.12)	2 (6.25)	-	-	16 (50)
Laminitis	15	2 (13.3)	5 (33.3)	-	1 (6.6)	1 (6.6)	2 (13.3)	11 (73.3)
Hoof crack	15	4 (26.6)	2 (13.3)	1 (6.6)	-	-	1 (6.6)	8 (53.3)
Cork screw hoof	17	3 (17.6)	4 (23.5)	-	1 (5.8)	1 (5.8)	-	9 (52.9)
White line disease	11	3 (27.2)	-	-	2 (18.1)	-	-	5 (45.4)
Miscellaneous	24	6 (25)	3 (12.5)	2 (8.3)	1 (4.16)	1 (4.16)	0	13 (54.1)
Grand Total	159	38 (23.9)	27 (16.9)	8 (5.03)	9 (5.6)	4 (2.51)	4 (2.51)	96 (56.6)

Figures in parentheses indicate percentage of animals.

REFERENCES

Collick, D.W. (1994). Correlation of laminitis and fertility. 8th International symposium on disorders of ruminant digit and International conference on bovine lameness, Canada,*pp.* 227-235.

Collick, D.W.; Ward, W.R. and Dobson, H. (1989) - Association between types of lameness and fertility. *Vet. Rec.. 125:* 103-106.

Dews, H.F.,(1968) - Some aspects of lameness in dairy herds. *NewZealand Vet. J. 26:* 147-148.

Eddy, R.G. and Scott, C.P. (1980) - Some observations on the incidence of lameness in dairy cattle in Somerset. *Vet. Rec. 106 :* 140-144.

Lucy,,S., Rowland, G.J. and Russell, A.M. (1986). The association between lameness and fertility in the dairy cows. *Vet. Rec. 118:* 628-631.

Moberg, G.P. (1991) - How behavioural stress disrupts the endocrine control of reproduction in domestic animals. *J. Dairy Sci. 74:* 304-311.

Nanda, A.S., Ward, W.R. and Dobson, H. (1989). Effect of Naloxone on estradiol induced LH surge in transported cows. *J. Repd. Fert. 87:*803-807.

Peelar,, E.J.; Otte, M.J. and Esslemont, R.J. (19994) - Interrelationships of periparturient diseases in dairy cows. *Vet. Rec. 134 :* 129-132.

Whittakar, D.A.; Kelly, J.M. and Smith, E.J. (1983) - Incidence of lameness in dairy cows. *Vet. Rec. 113:* 60-82.

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A Retrospective Study on Survivability and Fertility following Caesarean section in Bovines

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ABSTRACT

A retrospective study of the caesarean operations performed under field conditions and at Punjab Agricultural University clinics was conducted. The dam survival rate in dystocias due to foetal oversize and uterine torsion was significantly higher ($p<0.05$) in field as compared to PAU clinics cases, probably because of lesser degree of previous handling in the former. As the interval between the onset of first stage parturition and operation increased, both survival and conception rates decreased.

———x———x———

Caesarean operation in cattle is a feasible alternative to handle severe dystocia. In the available literature, most of the information about caesarean operations has come from institutional clinics (Kumar, 1990 and Prabhakar, 1995). Although such cases might have benefitted from high standards of asepsis and expertise, the outcome have been adversely affected because of delay in presentation and history of injudicious handling at the field level. In the present study attempt has been made to compare the success of caesarean section under field condition and at institutional clinics and to study the subsequent fertility following caesarean section.

MATERIALS AND METHODS

The information regarding caesarean operations being performed in field and at PAU clinics was collected through separate questionnaires. The questionnaires included the information regarding survival, post-operation cyclicity and conception. For field operated cases, 150 questionnaires were circulated among the leading veterinarians of different districts of punjab, who usually perform caesarean sections. Out of these, 73 responses were received back from 8 districts. One veterinarian reported 30 operations, four reported 14, 16, 15 and 10 operations and none of the others reported more than 4 operations. For PAU clinics cases, the details of 155 cases operated for caesarean section during the period 1991-94 were collected from the available records. Out of these records, complete addresses of 50 farmers, whose animals survived were taken and a questionnaire prepared in regional language (punjabi) was circulated among the farmers to extract information regarding future fertility; only 15 responses were received. For determining fertility rate, field and PAU clinics cases were pooled together owing to limited data. Statistical analysis of the data were done by using Student 's' 't' test (Gupta, 1986).

RESULTS AND DISCUSSION

Survival rate following caesarean section with respect to various causes of dystocia is presented in Table 1. Survival rate of dam after caesarean section in field operated cases (49 out of 73 : 67.1%)

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was higher than that at PAU clinics (66 out of 155 ; 43.2%). This might be due to lesser degree of previous handling in field cases (34.2%) as compared to PAU clinics (65.7 %). Singh (1991) found lower survival rate in injudiciously handled and delayed dystocia cases (50.6%) than fresh unattended cases (75.1.%). In the present study the survival rate was significantly higher ($p<0.05$) in dystocias due to foetal oversize and uterine torsion in field as compared to PAU clinics cases. Survival rate following caesarean section at PAU clinics have been reported to be low (25.0%) in buffaloes (Prabhakar, 1995), especially in cases of uterine torsion being 15.2 and 16.4 % as reported by Singla (1988) and Kumar (1990), respectively.

In both field and PAU clinics operated cases, as the time period between the first stage parturition and the operation increased,. the survival rate decreased gradually (Table.2). Caesarean section performed later than 18 to 36 hours led to high mortality rate probably owing to endotoxaemia (Top and Verdnock, 1979 and Saxena *et al*, 1989), uterine oedema, haemorrhage and/or dehydration (Roberts 1971). Saxena *et al* (1989) recorded 53.8% survivability in caesarean operated bovines suffering from dystocia for more than 36 hours.

Fertility status following caesarean section in relation to duration of dystocia has been shown inTable 3. Conception rate was lower in the animals in which the interval between the first stage of parturition and operation was more, probably because of high tendency of formation of uterine adhesions. The uterine adhesions play a major role in causing infertility after the caesarean operation (Roberts, 1971). In contrast Cattell and Dobson (1990) observed 75% conception rate after caesarean section in British cows in which elective surgery is usually performed in fresh cases as also evident from high calf survival rate (95%).

Table 1. Survival rate following caesarean section with respect to various causes of dystocia.

Indication for Operation	Field Cases			PAU clinics Cases		
	Total	Previously handled	Survival rate (%)	Total	Previously handled	Survival rate (%))
Foetal causes						
Oversize	10	3	70.0*	13	10	30.7
Monster	9	2	66.6	7	6	71.4
Maternal causes						
Uterine torsion	19	8	73.6*	69	44	33.0
Pelvic fracture/ Abnormal pelvis	13	4	46.1	20	11	60.0
Incomplete cervical dilatation	12	5	75.0	16	9	62.5
Others	10	3	70.0	30	22	40.0
Total	73	25 (34.2)	67.1	155	102 (65.7)	43.2

Figures in parentheses indicate percentage. *$p<0.05$ in a row

Table.2: Survival rate of caesarean operated animals in relation to duration of dystocia.

Duration of dystocia	Field cases		PAU clinics cases	
	Total	Survival rate (%)	Total	Survival rate (%)
Less than 36 hours	31	74.1	17	64.7
36 to 72 hours	26	57.6	87	50.5
More than 72 hours	16	43.7	51	35.2
Total	73	67.1	155	43.2

Table. 3: Fertility status of pooled field and PAU clinics cases operated for caesarean section in relation to duration of dystocia.

Duration of Dystocia	Total	Conceived	Not conceived	Status not known
Less than 36 hours	47	15 (31.9)	15 (31.9)	17 (36.2)
36 to 72 hours	18	5 (27.7)	6 (33.3)	7 (38.9)
72 to 108 hours	13	3 (23.0)	5 (38.5)	5 (38.5)
More than 108 hours	12	2 (16.6)	5 (41.7)	5 (41.7)
Total	90	25 (27.7)	31 (34.4)	34 (37.8)

Figures in parentheses indicate percentage

REFERENCES

Cattell, J.H. & Dobson, H. (1990).A survey of caesarean operations in cattle in general veterinary practice. Vet. Rec. *129:* 395-399.

Gupta, S.P. (1986). Statistical methods. 21st revised edn. Sultan chand and sons, New Delhi, India. P-125.

Kumar, P. (1990). Some studies on the cervical changes in the uterine torsion cases with special reference to cervical dilatation in buffaloes. M.V.Sc. Thesis submitted to Punjab Agri.Univ.Ludhiana, India.

Prabhakar, S. (1995). Studies on modulation of stress of dystocia in buffaloes. Ph.D. Thesis submitted to Punjab Agri. Univ. Ludhiana, India.

Roberts, S.J. (1971). Veterinary Obstetrics and Genital Diseases. 2nd edn.*P:* 262-168.

Saxena, O.P., Varshney, A.O., Jadon, N.S., Sharma, V.K. and Dabas, Y.P.S. 1989. Surgical management of dystocia in bovine : A clinical study. Indian. Vet. J.*66:* 562.

Singh, M. 1991. Studies on changes in blood and ruminal functions in buffaloes with dystocia. M.V. Sc Thesis submitted to Punjab Agri. Univ. Ludhiana, India.

Singla, V.K. (1989). Some studies on uterine torsion in buffaloes. M.V.Sc Thesis submitted to Punjab Agri. Univ. Ludhiana, India.

Top, W., & Verdnock, M. (1979). Handbook of Bovine Obstetrics. Williams land Wilkins, Baltimore, London. P-88.

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Clinical efficacy of MAU drug for induction of oestrus in postpartum anoestrus buffaloes

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ABSTRACT

The newly formulated durg based on the information from the Materia Medica of Ayurvedic Medicines was prepared and named as MAU drug. Six ayurvedic ingredients were selected, pulvarised and packed in Department of Gynaecology and Obstetrics, M.A.U. Parbhani. The administration of 5 gms of this formulation per buffalo as a single dose orally, effected 60 per cent induction of oestrus with mean interval of 14.08 days. The conception rate was found to be 60 per cent.

The anoestrus is major problem faced by the buffalo owners both in farm and field conditions. The main reason of anoestrus in rural buffaloes is due to nutritional deficiencies, as the farmers cannot meet the nutritional requirements of their herds due to their poor economical conditions. The service period, dry period and intercalving period are relatively long in buffaloes which is primarily due to postpartum anoestrus. Since the hormonal therapies for anoestrus is very costly, a non-hormonal herbal preparations are being largely employed in the treatment of postpartum anoestrus.

It is well known fact that traditional indigenous veterinary remedies are routinely practised in animal treatment particularly in rural areas. Standardisation of various pharmacologically and clinically proven drugs have already been initiated. The objective of the present study was to evaluate the efficacy of newly formulated drug on induction of oestrus in postpartum anoestrus buffaloes.

MATERIALS AND METHODS

A total of 30 rural postpartum anoestrus buffaloes were selected from village Pangali, under the jurisdiction of Livestock Development Officer (Extension), Panchayat Samiti, Parbhani. These buffaloes were having history of not exhibiting heat 90 days or above following parturition. Group I consisted of 20 buffaloes, which were administered single dose (50 gms) of MAU drug per buffalo orally. Group II consisted of 10 buffaloes, which were untreated. The animals under treatment and control group were observed regularly by per rectal examination for a period of 42 days post treatment for detection of a oestrus and related ovarian changes.

MAU drug is a ayurvedic product consisted of Ulatkambal 10 gms, Bambu leaves 10 gms, Jute seeds 16 gms, Lodhra 10 gms, Hirabol 2 gms and Kalabol or Korphad 2 gms per single dose pack. The above formulated ayurvedic ingredients were pulvarised and packed in Department of Gynaecology and Obstetrics, Veterinary College, M.A.U., Parbhani. The animals which responded to the MAU drug treatment with manifestation of ovulatory oestrus were bred naturally or artificially and the pregnancy was confirmed 90 days post breeding.

RESULTS AND DISCUSSION

Twelve out of twenty (60%) treated animals in group I exhibited oestrus, with mean time interval of 14.08 days. Subsequent to gynaeco-clinical examination ten buffaloes were bred, out of that 6 (60%) buffaloes conceived. All the ten buffaloes from untreated control group did not exhibit any clinical signs of oestrus nor any ovarian activity. Since MAU drug is a new formulation there are no earlier studies available regarding induction of oestrus and conception rate.

However, several other indigenous drugs marketed by Pharmacutial companies like Prajana (Patil *et al.,* 1983); Heatinee (Nemade *et al.,* 1994); Estrona (Shah and Darashri, 1985) and Moralac tablets (Dhoble and Markandeya, 1995) have been tried with regard to induction of oestrus and ovulation in diary cows and buffaloes.

Higher values of induction of oestrus and conception rate were reported by Patil *et al.,*. (1983) in cows, Shah and Derashri 1985) and Nemade *et al.,*. (1994) in buffaloes. The response of newly MAU drug for induction of oestrus was similar and conception rate was lower than those reported by Dhoble and Markandeya (1995). New formulation was found to be effective in treating the anoestrus cases with a single dose.

Based on the results of induction of oestrus and the time required for the manifestations of oestrus after administration of this treatment, it appears that MAU drug is fairly efficient in induction of oestrus in postpartum anoestrus buffaloes. However, there is need to further improve this formulation for its clinical efficacy in induction of oestrus, may be by addition of certain other related ingredients. Further there is also a need to study the pharmacodynamics of this formulation and its relationship with improvement of fertility.

REFERENCES

Dhoble, RL. and Markandeya,N.M. (1995). Clinical response to Morolac in postpartum anoestrus Deoni cows. Buffalo J. *11(2):* 170-181.

Nemade, P.K., Gatne, M.M.,Somkuwar, A.P. and Ranade, V.V. (1994). Oestrogenic activity of herbal preparations Heatinee in mice and buffaloes. The Veterinarian *18:* 9-11.

Patil, J.S., Bangalia, N.S., Sinha, A.K., Sharma, A.S. and Chopra, S.C. (1983) Clinical efficacy of Prajana, Lugol's paint and utero-ovarian massage in anoestrus cows. Indian Vet. J.*60:* 1019-1020.

Shah, I.M. and Derashri, H.J. (1985) Anoestrus in buffaloes: Treatment with Estrona. Indian J. Anim. Reprod.*6:* 81-83).

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Retained Foetal Membranes in Crossbred cows - Herbal Treatment and Uterine Involution

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ABSTRACT

Exapar was found to be effective in expelling the foetal membranes in about 50 per cent of crossbred cows having retention of foetal membranes (RFM) and also involution of uterus and occurrence of first post partum heat were earlier when compared to cows having RFM but treated with Replanta.

The impact of RFM on the farmers is great in that it causes great financial loss by way of increased intercalving periods due to delayed involution of uterus and uterine complications such as endometritis and pyometra leading to infertility or sterility. As there is dearth of information on the drug of choice for the expulsion of RFM, present study was undertaken to study the efficacy of two drugs in expulsion of foetal membranes, involution of uterus and occurrence of first post partum heat.

MATERIALS AND METHODS

In the present work twenty crossbred cows with RFM were selected from the clinics, College of Veterinary Science. Tirupati as well as from the Veterinary Hospitals in and around Chittoor. They were divided into two groups randomly and administered Exapar or Replata. Group I were treated with Exaper 100 ml twice on the first day and 50ml on the subsequent two days. Group II were treated similarly with Replanta 100 gm and 50 gms for three days.

The efficacy of Exapar and Replanta was evaluated on the basis of the expulsion of foetal membranes (FM) and involution of uterus as well as on the occurrence of first post partum heat. Twenty cows without RFM were utilized as control animals. The method suggested by Francis and Raa (1971) was adopted to study the uterine involution.

RESULTS AND DISCUSSION

Out of 10 cows having RFM treated with Exapar, 5 cows expelled the FM completely on the 2nd day. The other 5 cows did not shed FM evenafter 3 days which were removed manually without much difficulty. All the 10 cows having RFM treated with Replanta did not shed FM evenafter 3 days. When the attempts made to remove the same manually slight difficulty was felt. Contrary to the present observations Chakrabarthi and Pal (1990) and Hamidul Islam and Nooruddin (1990) found replanta as a drug of choice for the expulsion of FM in cows who have administered different combinations of Replanta (oral) along with I/u antibiotics, antiseptics and calcium borogluconate (I/V). This might be the reason for difference with the present study.

The mean size of the gravid and nongravid uterine horns from first week post

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* Forms part of Thesis for M.V.Sc submitted by the first author to Acharya N.G. Ranga Agricultural University, Hyderabad

Exaper - Dabur Ayurvet Ltd., Ghaziabad - 201 010.
Replanta - Indian Herbs, Bangalore 560 082

partum to 8 weeks in various groups is indicated in Table 1. In 10 cows without RFM and in 5 cows which expelled the FM after Exapar treatment, the involution of the gravid and nongravid uterine horns was completed by 5 weeks and 4 weeks after parturition, respectively. In 5 cows which did not expel FM after Exapar treatment and in 10 Replanta treated cows, the same was completed by 6 weeks and 5 weeks after parturition, respectively. In the cows treated with Exapar the mean time of involution was less when compared to those treated with Replanta (Table 1).

The mean time of involution of gravid and nongravid uterine horns between cows without RFM and cows having RFM treated with Replanta as well as between cows treated with Exapar and Replanta was significant ($p<0.01$) but in significant between cows without RFM and cows having RFM treated with Exapar. The present findings are similar to the findings of Morrow *et al.*, (1969) in cows with RFM and findings of Choudhury *et al.*, (1974). Agasti *et al.*, (1975) and Kumpf (1984) in cows without RFM. The difference between the cows without RFM and with RFM, might be due to the introduction of microorganisms while removing the foetal membranes as opined by Banerjee (1966).

Cows without RFM exhibited first post partum heat on an average by 36.50±0.97 days. The mean time of occurrence of first post partum heat in cows having RFM and treated with Exapar and Replanta was found to be 39.30±1.89 and 42.40±1.55 days respectively. A significant difference ($P<0.05$) in the mean time of occurrence of first post partum heat was observed between cows without RFM and RFM cows treated with Replanta but it was insignificant between cows without RFM and Exapar treated cows. Though there was a slight difference in the mean time of occurrence of first post partum heat between Exapar and Replanta treated cows, the same was insignificant. The time of occurrence of first post partum heat might be influenced by the time taken for the complete involution of uterus as opined by Paisldy *et al.*, (1986).

Table. 1: Involution of uterine horns in normally calved cows without RFM and in cows having RFM treated with Exapar and Replanta.

	Involution of gravid uterine horn				Involution of non-gravid uterine horn			
Weeks post-partum	Normally calved cows without RFM (cm)	Cows having RFM		Mean values for weeks (cm)	Normally calved cows without RFM	Cows having RFM		Mean values for weeks (cm)
		Treated with Exapar (cm)	Treated with Replanta (cm)			Treated with Exaper (cm)	Treated with Replanta (cm)	
1	$11.20^{a}\pm0.46$	$11.70^{a}\pm0.60$	$13.05^{a}\pm0.66$	11.98^{m}	$9.25^{a}\pm0.49$	$10.15^{a}\pm0.58$	$11.55^{a}\pm0.51$	10.32^{m}
2	$8.25^{b}\pm0.28$	$8.75^{b}\pm0.54$	$9.75^{b}\pm0.38$	8.92^{n}	$6.80^{b}\pm0.31$	$7.40^{b}\pm0.41$	$8.25^{b}\pm0.31$	7.48^{n}
3	$6.45^{c}\pm0.29$	$6.80^{c}\pm0.35$	$7.65^{c}\pm0.33$	6.97^{o}	$5.30^{c}\pm0.30$	$5.50^{c}\pm0.44$	$6.50^{c}\pm0.30$	5.77^{n}
4	$5.25^{d}\pm0.26$	$5.65^{d}\pm0.33$	$5.90^{d}\pm0.25$	5.60^{p}	$3.90^{d}\pm0.24$	$4.15^{d}\pm0.30$	$5.00^{d}\pm0.27$	4.35^{p}
5	$3.90^{e}\pm0.15$	$4.20^{e}\pm0.27$	$4.50^{e}\pm0.25$	4.20^{q}	$3.15^{de}\pm0.19$	$3.25^{e}\pm0.14$	3.85 ± 0.14	3.42^{q}
6	$3.15^{ef}\pm0.14$	$3.25^{f}\pm0.28$	$3.50^{f}\pm0.21$	3.30^{r}	$2.50^{ef}\pm0.14$	$2.60^{ef}\pm0.12$	$3.00^{ef}\pm0.10$	2.70^{r}
7	$2.65^{fg}\pm0.14$	$2.75^{fg}\pm0.22$	$3.00^{fg}\pm0.20$	2.80^{rs}	$2.40^{fg}\pm0.09$	$2.50^{fg}\pm0.07$	$2.60^{fg}\pm0.09$	2.50^{rs}
8	$2.40^{g}\pm0.14$	$2.45^{g}\pm0.17$	$2.75^{g}\pm0.13$	2.53^{s}	$2.30^{g}\pm0.08$	$2.35^{g}\pm0.07$	$2.40^{g}\pm0.06$	2.35^{s}
	5.41^{u}	5.69^{u}	6.26^{v}	5.79^{x}	4.45^{u}	4.74^{u}	5.39^{v}	4.86^{v}

Values with different superscripts in a column differ significantly ($P < 0.01$)

REFERENCES

Agasti, M.K., Choudhry, G., Banerjee, G.C. and Banerjee, T.K. 1975. Studies on certain aspects of uterine involution in Jersey X Haryana females folowing first calving. Indian Journal of Animal Science *45(9):* 186-188.

Banerjee, A.K. 1966. A study of the action ofTerramycine in the bacterial flora of the uterus in cattle following retained placenta. Indian Veterinary Journal *43:* 319-326.

Chakrabarthi A. and Pal,A. 1990. Therapeutic efficacy of Replanta against retained foetal membranes in crossbred cattle. Pashudhan 5-9-1990..

Choudhury, G., Agasti, M.K., Banerjee, G.C. and Ghosh, M.N. 1974. Studies on certain aspects of uterine invlutionin Holstein X Haryana females at first calving. Indian Veterinary Journal *51(6):* 395-400.

Francis, E.C. and Raja, C.K.S.V. 1971. A study on the genital involution in Sindhi cows. Kerala Journal of Veterinary Science *2(2):* 113-118.

Hamidul Islam, A.F.M. and Nooruddin, M. 1990. Treatment of some uterine disorders in bovines by Replanta. Pashudhan *5(8):* pp 4.

Kumpf, 1984. Course of the puerperal period in cows with placental retention as indicated by clinical chemical values in the blood. Inaugural Dissertation Fachbereich Veterinarmedizin Justus Leibig Universi.tat; Giessen 253 pp. Cited Veterinary Bulletin *55(11);* 1985 Abstract No. 7319.

Morrow, D.A., Roberts, S.J. and Mc Entee, K. 1969. Post-partum ovarian activity and involution of the uterus and cervix in dairy cattle. II. Involution of uterus and cervix. Cornell. Vet. *59(2):* 190-198.

Paisldy, L.G., Mickelsen, W.D. and Anderson, P.B. 1986. Mechanisms and therapy for retained foetal membranes and uterine infections of cows. A review. Theriogenology *25(2/3)L:* 353-381.

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Effect of intrauterine bacterial infusion Induced endometritis on bacterial count, oestrus cycle length and progesterone concentration in crossbred cows

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ABSTRACT

Eighteen crossbred cows were utilised to determine the effect of induced endometritis on bacterial count, Oestrus cycle length and progesterone concentration. Statistical analysis of bacterial count revealed highly significant difference ($P<0.01$) between follicular and luteal phase groups on day 2 and 5 of post bacterial inoculation. Induced endometritis increased the oestrus cycle interval from 21.33±0.33 days in control to 28.33±0.56 days in cows infused with bacterial culture during luteal phase of oestrus cycle. Where as oestrus cycle interval in experimental cows during follicular phase did not differ significantly between induced and control group of cows. Progesterone concentrations differed significantly between follicular phase and luteal phase cows. The study indicated that the uteri of folicular and luteal phase animals differ significantly in their inflammatory response to bacterial infusions.

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Reproductive efficiency is the major determinant of life time productivity in female livestock. Endometritis is a common reproductive disorder in dairy cattle (Coleman *et al.,* 1985). Endometritis increases intervals from calving to ovulation, detected estrus, and conception and it delays uterine and cervical involution (Lindell *et al.,* 1982, Fonseca *et al.,* 1983 and Coleman *et al.,* 1985). Therefore, the present study was undertaken to study the effect of induced endometritis on bacterial count, oestrus cycle length and progesterone concentration in cows.

MATERIALS AND METHODS

Eighteen crossbred cows, maintained at University Research Station, Madhavaram, were selected for experimental induction of endometritis. Cows were examined to eliminate clinical abnormalities of the reproductive tract and stage of Oestrus cycle was determined. Six crossbred cows were selected with luteal phase and bacterial inoculation was carried on 11th day of cycle. Another six cows with follicular phase of the cycle were selected and bacterial inoculation was done on the day of estrus. The control group comprised of six cows out of which 3 cows with follicular phase and 3 with luteal phase. They were administred with phosphate buffer saline.Corynebacterium pyogenes organisms with a strength of 3×10^9 colony forming units were infused into both uterine horns transcervically by using infusion glass pipettes. Bacteriological samples were collected aseptically from the uterus of all cows before commencement of experiment, second and fifth day following the inoculation. Blood samples were also

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Part of the Ph.D Thesis submitted by Senior author to Tamilnadu Veterinary and Animal Sciences University, Chennai-7.

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collected on the above mentioned days for estimation of progesterone in serum. All experimental animals were monitored for oestrus cycle evaluation by a teaser bull and also by rectal palpation (Vecchio *et al.,* 1992). Bacterial count was done by adopting plate count technique as described by Malik (1967). Progesterone concentration of serum samples was estimated by Coat-A-Count method of solidphase radioimmunoassay as described by Kubasik (1984).

RESULTS AND DISCUSSION

The mean bacterial count was $2.166\pm0.945\times10^6$ per ml and $25.666\pm1.580\times10^6$ after 2nd day of bacterial inoculation in experimental cows in follicular and luteal phase respectively. The bacterial count was nil on the 5th day of bacterial inoculation in cows in follicules phase group where as they were $43.500\pm1.540\times10^6$ per ml in luteal phase cows. The bacterial count differed significantly ($P<0.05$) between follicular phase and luteal phase animals on day second and fifth of bacterial inoculation. The difference in bacterial activity might be due to the action of progesterone which inhibits the uterine defence mechanism of luteal phase animals. This was in agreement with findings of Black *et al.,* (1954) and Hawk *et al.,* (1957). They recovered large number of bacteria from the uteri of luteal phase cows after inoculation, where as the uteri of follicular phase animals contained relatively few of introduced organisms. Nishikawa *et al.,* (1984) studied the effect of stage of Oestrus cycle on uterine infection induced by Escherichia Coli. They observed that Escherichia coli inoculated in dioestrus stage induced purulent endometritis, whereas Escherichia coli inoculated at proestrus stage did not cause purulent endometritis. They opined that stage of oestrus cycle affects the course of uterine infection. Hawk *et al.,* (1964) suggested that bactericidal activity was inhibited by progesterone.

The mean oestrus cycle length in control group of cows was 21.33 ± 0.33 days whereas cows infused with bacterial culture during luteal phase showed significantly ($P<0.01$) increased oestrus cycle length of 28.33 ± 0.56 days. The mean oestrus cycle length was 20.67 ± 0.42 days in cows infused with bacterial culture during follicular phase when compared to that of 20.33 ± 0.33 days in control group of cows. Vecchio *et al.,* (1992) observed that induced endometritis increased interestrus interval from 20.6 ± 1.0 day for control cows to 27.7 ± 1.0 day for the cows infused with bacteria.

The mean progesterone concentration was 0.15 ± 0.01 ng / ml in cows with luteal phase before bacterial infusions. The mean progesterone concentration on second day of bacterial inoculation was 0.21 ± 0.01 ng / ml in cows with luteal phase. The corresponding values on 5th day of bacterial inoculation were 0.65 ± 0.05 ng / ml in cows with follicular phase and 4.17 ± 0.11 ng / ml in cows with luteal phase. The high bacterial growth might be due to increase in levels of progesterone which inhibits the uterine defence machanisms of luteal phase animals. The results of the present study indicated that the uteri of follicular and luteal phase animals differ in their inflammatory response to infectious agents. This was in accordance with the findings reported by Mc Donald *et al.,* (1952), Black *et al.,* (1953) and Rowson *et al.,* (1953).

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REFERENCES

Black, W.G., Ulbeg, L.C. Kidder, H.E., Simon, T, McNutt, S.H. and Casida, L.E. (1953). inflammatory response of the bovine endometrium. Am.j. Vet Res., *14:* 179.

Black, W.G., Simon, J., Kidder, H.E. and Wiltbank, J.N. (1954). Bactericidal activity of the Uterus in the rabbit and the cow. Am. J. Vet. Res., *15:* 247.

Coleman, D.a. Thayne, W.V. and Dailey, R.A. (1985). Factors affecting reproductive performance of dairy cows. J. Dairy. Sci., *68:* 1793.

Fonseca, F.A., Britt, H.H., Mc Daniel, Wilk, J.C. and Raikes, A.H. (1983). Reproductive traits of Holsteins and Jerseys. Effects of age, milk yield and clinical abnormalities on involution of cervix and uterus. J. Dairy Sci., *66:* 1128.

Hawk, H.W., Simon, J., Mc Nutt, S.H. and Casida, L.E. (1957). Investigations on the endocrine - controlled defence mechanism of estrous and pseudopregnant rabbit uteri. Am. J. Vet. Res., *18:* 171.

Hawk, H.W., Briasfield, T.H., Turner, G.D., Whitemore, G.W. and Norcross, M.A. (1964). Effect of ovarinan status on induced acute inflammatory responses in cattle uteri. Am. J. Vet. Res., *25:* 362.

Kubasik, N.P. (1984). Evaluation of a direct solid-phase radioimmunoassay for progesterone, clin chem., *30:* 284.

Lindell, J.O., Klndahl, H., Janson L. and Edqvist, L.E. (1982) post partum release of prostaglandin F_2oc and uterine involution in the cow, Theriogenology, *17:* 237.

Malik, B.S. (1967). Laboratory manual for bacteriology and immunology. Ist 3d. British Book Depot, Lucknow.

Mc. Donald, L.E., Black, W.G., Mc Nutt, S.H., and CAsida, L.E. (1952). The response of the rabit uterus to instillation of Semen at different phases of the oestrous cycle. Am. J. Vet. Res. *13:* 419.

Nishikawa, Y., Baba, T. and Inori, T. (1984). Effect of the estrus cycle on Uterine infection induced by Escherichia coli. Infection and Immunity, *43:* 678. cited in Vet. Bull., *54:* 3087.

Rowson, L.E.A., Lamming, G.E. and Fry, R.M. (1953). The relationship between ovarian hormones and uterine infection. Vet. Rec., *65:* 335.

Vecchio, R.P. Del., Matsas, D.J. Inzana, T., Sponenberg, D.P. and lewis, G.S. (1992). Effect of intrauterine bacterial infusions and subsequent endometritis on prostaglandin F2 alpha metabolic concentrations in post partum beef cows. J. Amin. Sci. *70* 3158.

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Assessment of embryonic mortality in cows inseminated with post-thaw incubated semen

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ABSTRACT

Rectal examination of cows inseminated with 0 hr. 1 hr and 2 hr post-thaw incubation of semen in cotton, on day 21 and 35 (day 0 being the day of AI) for the presence or absence of CL in the ovary and on the day 60 for confirmation of pregnancy indicated 7.1, 8.3 and 21.4% incidence of embryonic mortality, the mortality being higher following 2 hr post-thaw AI interval.

———x———x———

It is generally accepted that while fertilization rates after artificial insemination are close to 90%, the calving rates are close to 50%. Fertilization rates of 82-95% have been reported following use of frozen thawed semen (Wishart and Young, 1974, Spitzer *et al.,* 1978; Shelton *et al.,* 1979; Diskin and Sreenan, 1980). However, embryo survival rates are considerably low. Pregnancy rates to single insemination are reported to be 50-57% (Sreenan and Mulvehill, 1975; Roche *et al.,* 1977).

The introduction of frozen semen technology in many geographical locations of our country has created new problems. In an attempt to extend AI to farmers' door the thawed semen straws from AI centres are some time carried to door step of cattle owner after being wrapped in cotton. This leads to considerable variation in post-thaw interval to AI depending upon the distance involved and transport facilities available.

The present study was aimed to study the effect of such practice on embryonic mortality in cows.

MATERIALS AND METHODS

A total of 75 cows belonging to the dairy farm of HP. Krish Vishvavidayala were employed in this study. They were divided into three groups each comprising of 25 Jersey cows. Cows in group I were inseminated within 10 min of thawing of straws. Cows in group II were inseminated with thawed semen wrapped in cottonwool for 1 hr at room temperature (circa 25°C), while cows in group III were inseminated with thawed semen wrapped in cotton for 2 hr. All these animals were examined per rectum on days 21 and 35, (Day 0 being the day of AI) for the presence or absence of corpus luteum (CL) in the ovary.The animals which possessed CL on both days on the same ovary at same place and were not found to be pregnant on day 60 were suspected to have undergone early embryonic mortality subsequent to maternal recognition of pregnancy.

RESULTS AND DISCUSSION

The data tabulated in Table indicate that of three groups of 25 animals each 14, 12 and 14 cows, respectively, possessed CL on days 21 and 35. Rectal examination on day 60 post AI revealed that 13 (52.0%). 11 (44.0%) and 11(44.0%) animals were pregnant in the three groups, respectively.

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Thus out of 14, 12 and 14 cows which possessed C.L. on day 21, 1, 1 and 3 animals which were not found pregnant on day 60 indicated 7.1, 8.3 and 21.4% incidence of early embryonic mortality in the three groups.

Apparently, the incidence of embryonic mortality in cows inseminated with semen incubated in cotton for 2 hr after thawing (Group III) was two-and-a-half to three-fold greater than in cows inseminated either within 10 min of thawing (group I) or after post thaw interval of 1 hr (group II). However, the difference could not be resolved in statistical terms presumable due to low population size. Henricks and associates (1971) showed a fertilization rate of 89% but the proportion of embryos surviving at day 42 after insemination was only 70%. Since these results indicate that the number of animals inseminated 2 hr. post-thaw (group III) was much higher than either the group I or II, a compromise in the management practice of inseminating with the semen upto 2 hr may lead to an increase in embryonic mortality.

Table: Embryonic mortality in cows inseminated with thawed semen stored in cotton at room temperature for different intervals.

Attributes		Hours Post-thaw (cotton incubation) 0 (Group I)	1 (Grtoup II)	2 (Group III)
No. inseminated		25	25	25
No. possessing CL (%)				
	Day 21	14(56.0)	12(48.0)	14(56.0)
	Day 35	14(56.0)	12(48.0)	14(56.0)
No. pregnant		13	11	11
Embryonic mortality rate (%)		1/14(7.1)	1/12(8.3)	3/14(21.4)

REFERENCES

Diskin, M.G. and Sreenan, J.M. (1980). Fertilization and embryonic mortality rates in beef heifers after artificial insemination. J. Reprod Fert.*59:* 463-478.

Henricks, D.M., Lamond, D.R. Hill, J.R. and Dickey, J.F. (1971). Plasma progesterone concentrations before mating and in early pregnancy in the beef heifer. J. Anim. sci *33:* 450-454.

Roche, J.F., Prenderville, D.J. and Davis, W.D. (1977). Calving rate following fixed time insemination after a 12 day progesterone treatment in dairy cows, beef cows and beifers. Vet Rec. *101:* 129-136.

Sheoton, J.N., Health, T.D., Old, K.G. and Turnbull, G.E. (1979). Non surgigal recovery of eggs from single ovulating bovines. Theriogenology *11:* 149-152

Spitzer, J.C., Niswender, G.D., Seidel, G.E. and Wiltbank, J.N. (1978) Fertilization and blood levels of progesterone and LH in beef heifers on a restricted energy diet. J. Anim. Sci *46:* 1071-2077.

Sreenan, J.M. and Mulvehill, P. (1975). The application of long and short term progestagen treatments for oestrous cycle control in heifers. J. Reprod. Fert. *45:* 867-869.

Wishart, D.F. and Young I.M. (1974) Artificial insemination of cattle at a predetermined time following treatment with a potent progestagen (Sc-21009). Vet. Rec. *95* 503-508.

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Studies on Certain Blood Constituent Profiles During Pregnancy in Indigenous Pigs of Assam

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ABSTRACT

An experiment was conducted to study the serum calcium, inorganic phosphorus, total protein, cholesterol and alkaline phosphatase levels, during pregnancy in indigenous pigs of Assam. Except calcium, the other blood constituents were found to be higher ($P<0.01$) in oestrus than prepubertal stage. During pregnancy, the levels of different blood constituents were found to be incressed towards the late pregnancy indicating the demands of these constituents by the foetus.

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During pregnancy, a sequence of changes occur including the biochemical constituents of blood. Protein as a nutrient is an essential components for both the dam and the growing foetus (West and Todd, 1967). The fluctuations of these constituents of blood indirectly reflects the demand and utilization by the foetus, dam or both. Although several scientists have worked on different constituents of blood during different stages of pregnancy, no information is available on the constituents of blood during different stages of pregnancy in indigenous pigs of Assam. Therefore, the present experiment was carried out.

MATERIALS AND METHODS

Twenty apparently healthy gilts approaching puberty were selected. The age of the animals ranged from 186-200 days on the beginning of the experiment and their body weight varied from 23 to 29 kg. All the animals were kept indoor and reared under standard managemental practices of the farm. The experimental animals reached puberty on 233.64±4.48 days and they were meted with a fertile boar in the third cycle. Blood samples were collected from each of experimental animals at the beginning of the experiment, on the day of oestrus, early pregnancy (30 days), mid pregnancy (60 days) and late pregnancy (90 days) Serum was separated from the blood samples and stored at -20° C till analysis was carried out. Calcium, inorganic phosphorus, total protein, cholesterol and alkaline phosphatase were estimated from the serum samples by adopting standard biochemical procedures. The data were analyzed statistically as per standard methods of Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Serum calcium, inorganic phosphorus, total protein, cholesterol and alkaline phosphatase during different stages of pregnancy in pigs are presented in Table 1. From the perusal of the table it is revealed that these blood constituents differed significantly ($P<0.01$) among different stages of pregnancy. The serum calcium levels did not differ significantly between the prepuberal and oestrus conditions. However, the levels of calcium rose significantly ($P<0.01$) towards the late pregnancy. Similar findings were also

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reported by various workers (Mogens, 1970; Yakimchuk, 1978). The lower level of serum calcium found during oestrus might be related with high concentration of oestrogenic hormone (Folley, 1936) in sows. However, the higher level of serum calcium found during pregnancy might be related with efficient absorption of calcium due to increase demand of calcium during pregnancy and lactation (Mogens, 1970).

The serum inorganic phosphorus was significantly ($P<0.01$) higher in oestrus, then it dropped during early pregnancy but increased towards the late pregnency. This might be associated with high level of oestrogenic activity which usually elevated the serum inorganic phosphorus level in circulation (Horvath and Kutas, 1959).

The serum protein level was highest in oestrus than prepubertal and pregnancy. With the advancement of pregnancy, the serum protein level increased which might be due to hike in oestrogen (Mehta *et al.*, 1989). McDonald (1980) reported that as the pregnency advanced, protein concentration in maternal circulation increased and indicating a positive association with pregnancy establishment. Increase of protein concentration during mid pregnancy may be explained as increased demand of protein by growing foetus and that for late pregnancy may be due increase synthesis of globulin action essential for colostrum synthesis (Larson, 1958).

A significant increase in serum cholesterol was noted in oestrus than prepubertal stage, which might be due to peak level of oestrogen. Physiological stress condition during oestrus might elevate the total serum cholesterol level (Searcy, 1969). During stress, the levels of catecholamines reported to be increased which might enhance the lipolysis of fat resulting increased levels of serum cholesterol (Bartley, 1970). The cholesterol levels again increased towards mid and late pregnancy might be due to increase in level of progesterone in the circulation (Jadhav *et al.*, 1977).

Higher level of serum alkaline phosphatase during oestrus than prepubertal stage might be due to peak level of oestrogen (Searcy, 1969). The mean value of serum alkaline phosphatase increased from oestrus to mid pregnancy followed by a decline in late pregnancy. The gradual increase in serum alkaline phosphatase level appeared to be due to liberation of this enzyme from the placenta (Boyer, 1961). Another possible explanation could be greater need of phosphate for implantation to occur (Mehta *et al.* 1989). Decreased level of serum alkaline phosphatase during late pregnancy may be due to utilization of this enzyme based material for growing foetus (Pathak *et al.*, 1986).

Table 1. Serum calcium, inorganic phosphorus, total protein, cholesterol and alkaline phosphatase during different stages of pregnancy in indigenous pigs of Assam.

Phases	Calcium mg%	Inorganic phosphorus mg%	Total protein g%	Cholesterol mg%	Alkaline phosphatase K.A.U./ml
Prepubertal	$9.66^{a}\pm0.23$	$6.14^{a}\pm0.15$	$6.04^{a}\pm0.12$	$94.89^{a}\pm2.59$	$7.06^{a}\pm2.31$
Oestrus	$9.31^{a}\pm0.24$	$7.08^{b}\pm0.10$	$7.86^{b}\pm0.10$	$119.46^{bc}\pm1.93$	$10.24^{b}3.13$
Early pregnancy	$10.07^{a}\pm0.18$	$5.67^{c}\pm0.18$	$6.94^{c}\pm0.16$	$100.26^{a}\pm3.32$	$10.68^{b}\pm2.64$
Mid pregnancy	$10.68^{c}\pm0.12$	$5.70^{c}\pm0.18$	$7.06^{c}\pm0.20$	$117.71^{bc}\pm2.58$	$11.96^{c}\pm2.81$
Late pregnancy	$10.76^{c}\pm0.24$	$6.44^{ad}\pm0.16$	$7.34^{bc}\pm0.18$	$122.46^{c}\pm3.13$	$11.26^{c}\pm3.24$

Figures bearing different superscripts in a column differ significantly ($P<0.05$).

REFERENCES

Bartley, J.C. (1970). Lipid metabolism. IN chemical Biochemistry of Domestic Animals. 2nd ed. Edited by Kaneko, J.J. and cornelius, C.E.; Academic press, New York.

Boyer, S.M. (1961). Science *134:* 1002 (Cf. Searcy, L. Ronald, 1969) Diagnostic Biochemistry, McGraw Hill Book Company, New York.

Folley, S.J. (1936). Nature *137:* 741. (Cf. Cornelius, C.E. and Kaneko, J.J. (1970). Clinical Biochemistry of Domenstic Animals. 2nd Edn. Academic press, New York.

Horvath, G. and Kutas, E. (1959). Acta. Vet. Acad. Sci. Hung. *9:* 183 (Cf. Cornellus, C.E. and Kaneko, J.J., 1970). Clinical Biochemistry of Domestic Animals. 2nd edn. Academic press, New York.

Jadhav, S.S.; Velhankar, D.P. and Deshpande, B.R. (1977). Variation in the serum cholesterol levels of Gir cows during pregnancy, perturition and puerperium. Indian Vet. J. *54(7):* 536-540.

Larson, B.L. (1958). J. Dairy Sci. *41:* 1033-1044 (Cf. Pathak, M.M.; Patel, A.V.; Diwan, Kalpana and Janakiraman, K. (1986). Blood serum protein at different stages of pregnancy in Surti Buffalo. Ind. J. Anim. Reprod. *7(1):* 66-69.

McDonald, I.E. (1980). Veterinary Endocrinology and Reproduction. 3rd Edn. Lea and Febiger, Philadelphia.

Mehta, J.S.; Bhatia, J.S.; Kohli, I.S.; Pareek, P.K.; Bishnei, B.L. and Gupta, A.K. (1989). Studies on serum alkaline phosphatase and protein in various reproductive states in cow. Ind. J. Anim. Reprod. *19(2):* 138-140.

Mogens, G.S. (1970). Calcium, inorganic phosphorus and magnesium metabolism in health and disease. P. 322 (Cf. Cornelius, C.E. and Kaneko, J.J. (1970) Clinical Biochemistry of Domestic Animals. 2nd edn. Academic press, New York.

Pathak, M.M.; Patel, A.V.; Diwan, Kalpana and Janakiraman, K. (1986). Blood serum alkaline phosphatase and peroxidase in pregnant Surti Buffalo. Ind. J. Anim. Reprod. *7(2):* 44-47.

Searcy, L. Ronald (1969). Diagnostic Bio-chemistry. McGraw Hill Book Company, New York.

Snedecor, G.W. and Cochran, W.G. (1967). Statistical Methods. 6th edn. The Iowa State University press, Iowa.

West, E.S. and Todd, H.S. (1967). Text Book of Biochemistry, 4th Edn. The McMillan Com, New York.

Yakimchuk, N.V. (1978). Biochemical composition of blood of sows. Veterinariya, Moscow. *8:* 94-95 (Cf. Vet. Bull. *49:* 2292).

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Effect of Sheep Follicular Fluid on the Maturation of Oocytes of Sheep in Vitro*

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ABSTRACT

The oocytes recovered from the ovaries of slaughtered animals were matured for 24 to 36h at 39° C under 5% CO_2 in air in TCM-199 medium supplemented with hormones, including FSH (5μg/ml), LH (10μg/ml) and estradiol-17β (1μg/ml) or sheep follicular fluid (SFF) recovered from ovarian follicles (3 to 5mm) or with combination of SFF and hormones. It was observed that inclusion of SFF along with hormones significantly increased the maturation rate (77.82%) when compared with medium alone (40.95%), medium with hormones (54.83%) and medium with SFF (66.95%). The results of this study shows that addition of SFF to the maturation medium can enhance the maturartion of oocytes of sheep *in vitro*.

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Recently, several studies have claimed a beneficial effect of inclusion of follicular fluid in medium for *in vitro* maturation of oocytes and their subsequent fertilization and development (Naito *et al.*, 1988, 1989; Yoshida *et al.*, 1992). However, pig follicular fluid has been shown to inhibit oocyte maturation (Stone *et al.*, 1978; Tsafriri *et al.*, 1982). The present study was conducted to examine the effect of sheep follicular fluid on the maturation of oocytes of sheep *in vitro*.

MATERIALS AND METHODS

Ovaries of sheep were obtained from Karnataka animal food corporation (KAFCO) slaughter house, Bangalore and were transported to the laboratory in sterile Dulbecco's phosphate buffered saline (DPBS) or normal saline with antibiotics at 37°C.

The follicular oocytes were collected aseptically from the ovaries by follicular aspiration or puncturing of follicles or slicing of ovaries or by slicing the aspirated ovaries into the collection medium (DPBS) supplemented with 5 per cent fetal calf serum (FCS). Oocytes with intact and tight cumulus cells were collected and washed three times in TCM-199 supplemented with 20% FCS and then cultured in groups of 10 to 15 oocytes in 50μl drops of specified maturation medium under mineral oil for 24 to 36 hrs at 39°C with 5% CO_2 under high humidity in a CO_2 incubator.

The experiment was conducted using TCM-199 medium supplemented with 10 per cent sheep follicular fluid (SFF) alone or medium supplemented with 10% SFF and hormones (5μg/ml FSH, 10μg/ml LH and 1μg/ml estradiol-17β) or medium with hormones for *in vitro* maturation and were compared with those results obtained using medium alone.

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Sheep follicular fluid (SFF) was aspirated from follicles having a diameter of 3 to 5mm. Aspirates were pooled and centrifuged at 3000 rpm for 30 minutes at room temperature, heat inactivated and filtered using 0.20μ filter and stored in one to two ml aliquots at - 20° °C until use.

The maturation of oocytes were assessed by their morphological changes and nuclear changes. The morphological changes were assessed by the expansion of cumulus oophorus cell mass, increase in perivitelline space, appearance of first polar body and breakdown of cortical granules. These changes were observed using inverted microscope (*Nikon* Diaphot TMD) at a magnification of 14,000 to 28,000 times. The nuclear maturation of oocytes were assessed by fixing of oocytes with aceto-methanol (1:3) and staining with 4 per cent Giemsa stain. The maturation was assessed on observing Metaphase-II chromosome patterns.

The statistical analysis was carried out as described by Snedecor and Conchran (1988).

RESULTS AND DISCUSSION

The rate of matauration of oocytes of sheep matured *in vitro* in TCM-199 medium supplemented with SFF, medium with only hormones or medium alone were examined and results are presented in Table 1.

The maturation of oocytes of sheep matured *in vitro* in the medium supplemented with SFF and hormones was 77.82±2.69 per cent which was significantly higher when compared to other supplements to the medium or medium alone. Out of the oocytes matured in medium with SFF and hormones, 73.25±0.83, 70.34±1.49 and 56.39±2.31 per cent of oocytes showed cumulus cell expansion (CCE), breakdown of cortical granules and increase in perivitelline space (PVS) respectively.

Further, addition of SFF alone to media showed significantly lower ($P \leq 0.05$) rate of maturation (66.95±1.28%), cumulus cell expansion (62.33±1.55%), breakdown of cortical granules (59.09±1.62%) and increase in PVS (50.64±1.05%), than those exposed to the medium containing SFF and hormones. Similarly in medium TCM-199 with hormones and in medium TCM-199 alone significantly lower percentage of maturation, cumulus cell expansion, breakdown of cortical granules and increase in perivitelline space were noticed.

The results of the present study shows that SFF induced the overall maturation rate (66.95±1.28%) in sheep oocytes *in vitro*, which demonstrated that homologous follicular fluid derived from ovarian follicles exert a stimulatory effect on *in vitro* maturation of oocytes.

In the present study the number of sheep oocytes matured under similar condition was lower than reported by Larocca *et al.,* (1993). This may be due to the low per cent of SFF added to the basic medium (10%) when compared to use of 30 per cent follicular fluid by Larocca *et al.,* (1993). Further, the results obtained from different species may not truly reflect a comparison of the effect of follicular fluids.

Further, it has been reported that the follicular fluid obtained from the different size follicles may also influence the rate of maturation of sheep oocytes *in vitro* (Sun *et al.,* 1994). Such a variation in rate maturation induced by follicular fluids from various sized follicles may account for the variations in the levels of hormones and growth factors present in the follicles of various sizes. In the present study, SFF was obtained by pooling the follicular fluid from

follicles of all sizes and incorporated into the medium at the rate of 10% as against 20 or 30 percent of follicular fluid incorporated by the earlier workers, which may be one of the reasons for lower rate of maturation obtained in present study.

The results suggested that the growth factors and hormones present in the SFF alone may be insufficient to induce *in vitro* maturation in the greater population of oocytes of sheep and further, the addition of hormones to the medium along with SFF enhance the rate of their maturation *in vitro*.

Table 1. Effect of supplementation of sheep follicular fluid (SFF) with or without hormones on *in vitro* maturation of oocytes of sheep

Experimental Protocals	No. of oocytes cultured	No. of oocytes matured	Percentage of matruration (Mean ± S.E.)	% Cumulus cell expansion (Mean ± S.E.)*	% Breakdown of CGS (Mean ± S.E.)*	% Increase in PVS (Mean ± S.E.)*
TCM-199 alone	188	77	40.95±2.06^{d}	35 (45.45±2.52)^{d}	32 (41.55±1.59)^{d}	14 (18.18±1.05)^{d}
TCM-199 + hormones	217	119	54.83±2.07^{c}	64 (53.78±2.04)^{c}	60 (50.42±1.79)^{c}	54 (45.37±0.77)^{c}
TCM-199 + SFF	230	154	66.95±1.28^{b}	96 (62.33±1.55)^{b}	91 (59.09±1.62)^{b}	78 (50.64±1.05)^{b}
TCM-199 + SFF + Hormones	221	172	77.82±2.69^{a}	126 (73.25±0.83)^{a}	121 (7.34±1.49)^{a}	97 (56.39±2.31)^{a}

Note: Means bearing any one common superscript in columns do not differ significantly with each other * Calculated from number of oocytes matures; PVS: Perivitelline space; CGS: Cortical granules.

REFERENCES

Larocca, C., Kmaid, S. and Calvo, J. (1993). Effect of follicular fluid and estrus cow serum and maturation, fertilization and development of the bovine oocytes *in vitro*. Theriogenology., *39 :* 253(abstr)/

Naito, K., Fukuda, Y. and Toyoda, Y. (1988). Effect of procine follicular fluid on male pronucleus formation in porcine oocytes matured *in vitro*. Gamate Research., *21 :* 289-295.

Naito, K., Fukuda, Y. and Ishibashi, I.(1989). Developmental ability of porcine ova matured in procine follicular fluid and fertilized *in vitro*. Theriogenology., *31 :*

Snedecor, G.W. and Cochran, W.G. (1988). Statistical methods. Oxford and IBH publishing CO. New Delhi, India.

Stone, S.L., Promerantz, S.H., Schwartz-Kripner, A. and Channing, C.P. (1978). Inhibitor of oocyte maturation from porcine follicular fluid : further purification and evidence for reversible action. Biol. Reprod., *19 :* 585-592.

Sun, F.J., Holm. P., Irvine, B., and Seamark, R.F. (1994). Effect of sheep and human follicular fluid on the maturation of sheep oocytes *in vitro*. Theriogenology., *41 :* 981-988.

Tsafriri, A., Dekel, N. and Bar - Ami, S. (1982). The role of oocyte maturation inhibitor in follicular regulation of oocyte maturation. J. Reprod. Fert., *64 :* 541-551.

Yoshida, M., Ishizaki, Y., Kawagishi, H., Bamba, K. and Kojima, Y. (1992). Effect of pig follicular fluid on maturation of pig oocytes *in vitro* and on their subsequent fertilizing and development capacity *in vitro*. J. Reprod. Fert., *95 :* 481-488.

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Successful Cryopreservation of Goat Embryos by Vitrification

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ABSTRACT

Ten adult cyclic goats were superovulated with 750 IU PMSG, 5 mg PGF_2 alpha and 1500 IU HCG The mean ovulation rate following laparotomy on day 6 post onset of oestrus was 10.90±1.52. A total of 140 embryos were recovered of which 47 and 72 were normal morula and blastocysts, respectively. Recovered embryos were cryopreserved by vitrification in a mixture of 25% glycerol and 25% 1,2-propanediol in modified Dulbeccos phosphate buffered saline (FBS). Twelve morphologically normal post-thaw vitrified embryos transferred to six recipients resulted into 66.67 per cent pregnancy rate with 50.0 per cent recipient kidded. The percentage of kids born in recipient-wise following transfer of post-thaw vitrified embryos was 50.0 per cent and kids born in transferred embryo-wise was 25.0 per cent.

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In embryo transfer practice, controlled slow freezing was routinely used for cryopreservation of mammalian embryos. Due to certain disadvantages of slow freezing method viz. higher cost., time consuming and injury caused to embryos due to formation of ice crystals, a rapid cryopreservation method, called vitrification, was developed (Ralland Fahy, 1985). This method eliminated potential damage to the embryos due to the formation of ice. However, only a few attempts (Yuswiati and Holtz, 1990 and Agrawal *et al.*, 1994) have been made to study the survivability of vitrified goat embryos following transfer to recipients. The present work has been undertaken to record the results of transfer of vitrified goat embryos to recipients.

MATERIALS AND METHODS

Ten adult cyclic (aged 1 to 2 years) healthy Assam Local goats were superovulated with 750 IU PMSG (Folligon, Intervet, Holland) each intramuscularly on day 9 to 11 of the oestrous cycle followed by 5mg PGF_2 alpha (lutalyse, Upjohn, USA) intramuscularly at 24 hour post-PMSG injection and 1500 IU HCG (Chorulon Intervet, Holland) intravenously at 6 hours post onset of oestrus. Oestrus was observed at 6 hour interval following the treatment and detected by a vasectomised buck. Receptive dose were allowed to mate naturally with a proven buck until they were no longer receptive. Lapratomy was performed on day 6 post onset of oestrus for monitoring ovarian response and embryo collection. Each uterine horn was flushed with sterile 15-20 ml Dulbecco's Phosphate Buffered Saline (PBS) enriched with glucose and anitbiotics and supplemented with 20% heat inactivated oestrus goat serum (HIGS) (Yuswiati and Holtz, 1990).

Following recovery, embryos were washed three times using fresh holding medium (PBS plus 20% HIGS). Morphological evaluation of embryos and quality grading, were conducted according to Lindner and Wright (1983). Embryos at

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the early morula to blastocyst stage with quality grade of excellent, good or fair were subjected to cryopreservation by vitrification according to the method of Massip *et al.*, (1987) and Yuswiati and Holtz (1990). Briefly, embryos were placed into modified PBS containing 10% glycerol (1.4 M) and 20% 1,2-propanediol (2.7 M) for 10 minutes at room temperature (20-27°C). After an equilibration period of 10 minutes embryos were exposed to precooled (4°C) vitrification solution (a mixture of 25% glycerol (3.4 M) and 25% 1,2-propanediol (3.4M) in modified PBS). Immediately embryos (1 or 2 per straw) were aspirated into a 0.5 ml French straw preloaded with 1.0M sucrose diluent in modified PBS and vitrification solution separated by air bubbles. The straw was then immediately sealed with polyvinyl alcohol powder and placed in liquid nitrogen vapour for 30 seconds and then immersed into liquid nitrogen (-196°C). After a storage period of more than 1 day, straws were thawed in a water bath at 20°C and the contents were emptied into a sterile watch glass containing 1.0M sucrose diluent in modified PBS. After 10 minutes, embryos were washed twice by placing them into modified PBS for 10 minutes at a time. Embryos were then assessed under stereozoom microscope for their morphological appearence and grouped as normal and abnormal embryos as per the criteria described by Hafez (1987).

Twelve morphologically normal post-thaw vitrified embryos were surgically transferred to six adult recipients 6 day after the end of a natural oestrus. The embryos were transferred to anterior part of the uterine horn ipsilateral to the ovary having atleast one corpus luteum. Recipient animals were closely observed for onset of oestrus. Animals which did not return to oestrus during 45 days post transfer of vitrified thawed embryos were considered pregnant and allowed to progress to term.

RESULTS AND DISCUSSION

The mean ovulation rate in doner animals were 10.90±1.52. This value was in close conformity with the findings of Armstrong and Evans (1983) and Sarmah *et al.*, (1996) in goats. Out of 140 number of embryos recovered, the incidence of blastocyst stage of embryos were the highest (72 nos) followed by morula (47 nos) at recovery on day 6 post-onset of oestrus. The observation made in the present study was similar to that of Nuti *et al.*, (1987) and Gogi (1993) in goats.

The pregnancy rate following transfer of post-thaw vitrified embryos to adult recipients was 66.67 per cent (4/6). Again the percentage of recipient kidded following transfer of post-thaw vitrified embryos was 50.0 per cent (2/4). Similar findings was also reported by Agrawal *et al.*, (1994) in goat with higher percentage of recipients kidded following transfer of post-thaw vitrified embryos.

The percentage of kids born in recipient-wise following transfer of post-thaw vitrified embryos was 50.0 per cent (3/6) and kids born in transferred embryo-wise was 25.0 per cent (3/12). Out of 3 kids born, one pregnant recipient delivered one male and one female, and the other recipient delivered one female kid with good health. The average birth weight of kids was 1.9 kg. The kidding rate in recipient-wise was higher than that of Yuswiati and Holtz (1990) and Agrawal *et al.*, (1994) in goats. The kidding rate in transferred embryo-wise recorded in the study was higher than that of Yuswiati and Holtz (1990) and Agrawal *et al.*, (1994) in goats, but was close to the findings of Schiewe *et al.*, (1991) in sheep. The higher

kidding rate obtained in the present study might be due to the transfer of normal post-thaw vitrified embryos to recipients with favourable uterine milieu which might have contributed in overcoming the stress of vitrification of embryos. Similar views were expressed by Mahmoudzaden *et al.*, (1993).

The present study revealed that successful pregnancy and kidding could be achieved following transfer of cryopreserved embryos by vitrification and one-step dilution.

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REFERENCES

Agrawal, K.P., Srivastava, C., Sharma, N.and Goel, A.K. (1994). A kid born by transfer of vitrified embryos in goat. Ind.J. Anim. Reprod. *15:* 102-103.

Armstrong, D.T. and Evans, G. (1983). Factors influencing success of embryo transfer in sheep and goats. Theriogenology. *19:* 31-42.

Gofoi, A.K. (1993). Study on certain aspects of embryo transfer technology with special reference to cryopreservation of embryos in Assam local goats. M.V.Sc. Thesis submitted to Assam AGril. Univ. Guwahati.

Hafez, E.S.E. (1987). Reproduction in Farm animals, 5th Ed. Lea & Febiger, Philadephia.

Lindner, G.M. and Wright, R.W. (1983). Bovine embryo morphology and ovulation. Theriogenology. *20:* 407-416.

Mahmoudzaden, A.R., Van Soons, A., Van Vlaenderen and Dekresf, A. (1993). A comparative study of the effect of one step addition of different vitrification solution on *in vitro* survival of vitrified bovine embryos theriogenology, *39:* 1291-1302.

Masspi, A., Van Der Zwalnsen, P. and Ectens F. (1985). Recent progress in cryopreservation of cattle embryos. Theriogenology. *27:* 69-79.

Nuti, L.C., Minhas, B.S., Baker, W.C., Capehart, J.S. and Marrack, P. (1987). Superovulation and recovery of zygotes from Nubian and Alpine dairy goats. Theriogenology. *28:* 481-488.

Rall, W.F. and Fahy, G.M. (1985). Ice-free cryopreservation of mouse embryos at-196°C by vitrification. Nature. *313:* 573-575/

Sarmah, B.K., Sarmah, B.C. and Chakravarty, P. (1996). Prospect of embryo transfer in local goats of Assam. Int. J. Anim. Sci. *11:* 181-182.

Schiewe, M.C., Rall, W.F., Stuart, L.D. and Wildt, D.E. (1991). Analysis of cryoprotectant, Cooling rate and in situ dilution using conventional freezing or vitrfication for cryopreserving sheep embryos. Theriogenology. *86:* 279-293.

Yuswiati, E. and Holtz, W. (1990). Successful transfer of vitrified goat embryos. Theriogenology. *34:* 629.632.

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Biometrics of Ovarian Oocytes and Embryos of Local Goats of Assam

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ABSTRACT

A biometrical study on 200 follicular oocytes collected from 30 pairs of ovaries at slaughter and on 34 normal flushed embryos (1-cell to late blastocyst) from local goats of Assam was carried out. The mean diameter of oocytes and embryos including and excluding the zona pellucida, the mean thickness of zona pellucida and the width of perivitelline space were studied.

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Information on morphological development and evaluation of female gametes and embryos helps in successful application of embryo transfer technology (ETT) in livestock production. Although there are reports on menturation characteristics of ova of cattle, pig, sheep and mouse, literature pertaining to the caprine embryo is scanty (Cole and Cupps, 1977; Salisbury *et al.*, 1985; Hafez, 1987). Therefore, the present study was undertaken to record the menturation characteristics of follicular oocytes and early embryos (1-cell to late blastocyst) of local goats of Assam.

MATERIALS AND METHODS

A total of 30 pairs of ovaries (right and left) of local goats of Assam were collected immediately after slaughter from local abattoirs. The ovaries were placed in a polythene bag and brought to the laboratory in a thermosflask containing ice cubes. The visible follicles of different sizes *viz.*, 5 mm, 3 mm, 2 mm and <2mm diameter were dissected out from the ovaries and placed in a petridish containing phosphate buffered saline (PBS). The oocytes were freed by repturing the follicles and then made free of excess granulosa cells. A total of 200 oocytes were considered for biometrics.

For biometrics of early embryos, a total of 34 morphologically normal embryos were collected from superovulated local goats of Assam by flushing the fallopian tubes and uterine horns with PBS enriched with BSA fraction V.

After collection, identification and evaluation, the folicular oocytes and superovulated embryos were subjected to the various measurements *viz.*, diameter of oocytes including zona pellucida, excluding zona pellucida, diameter of embryos including zona pellucida, diameter of cellmass of embryos, thickness of zona pellucida and width of perivitelline space. The measurements were taken under phase contrast microscope using an occular micrometer at 150X magnification following the procedure of Linares and King (1980).

RESULTS AND DISCUSSION

The measurements of follicular oocytes of local goats of Assam (Table 1) indicated that the mean diameter of oocytes including zona pellucida varied from 156.82±1.06μ to 168.70±1.90 μ and from 139.09±2.19μ to 148.12±1.42μ respectively. The mean diameter of embryos including zona pellucida and the mean diameter of cell mass were seen to vary from 162.19±1.92μ to 173.87±1.56μ and from 134.95±3.23μ to 153.11±1.56μ respectively (Table 2).

However, the overall mean diameter of oocytes and embryos was almost similar but biometrics of the oocytes of larger follicle, blastocyst and late blastocyst had a tendency to show higher measurements. the present findings of oocyte measurements are almost similar to the findings of Singh and Sharma (1992) while much wider range of diameter of embryos was also reported by Mapletoff (1986). The results pertaining to the mean thickness of zona pellucida of follicular oocytes varied from 9.86±0.33μ to 10.49±0.50; μ. Nearly same thickness of zona pellucida was observed in superovulated embryos varying from 10.38±0.00μ to 11.68±0.58μ respectively. The width of the perivitelline space of embryos (Table 2) had shown some concomitant variation with the change of the mean diameter of the cellmass. However, the width of the perivitelline space was not found in some cases with compact cell mass while in others it was 3.89±1.92μ

The difference in various dimensional characteristics in the morphology of caprine oocyte and embryos could be due to the follicular and oocyte maturation. The present overall findings were lower than that in cattle, sheep and pig (Linares and King, 1980; Salisbury *et al.*, 1985; Mapletoff, 1986) While Bonia (1992) reported nearly similar results in goat.

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Table 1. Measurements (Mean±SE) of oocytes of local goats of Assam

Sizes (diameter) of follicles (mm)	Diameter of oocytes		Thickness of zona pellucida (μ)
	Including zona pellucida (μ)	Excluding zona pellucida	
>5 (50)	168.70±1.90	148.12±1.42	10.49±0.05
4 (60)	165.46±1.11	145.97±1.64	10.38±0.00
3 (40)	165.43±1.56	145.69±1.26	10.32±0.06
2 (30)	160.71±1.79	139.96±1.87	9.95±0.24
>2 (20)	156.82±1.06	139.09±2.19	9.86±0.33

SE = Standard error

Figures in parentheses indicate number of observations.

Table 2. Measurements (Mean ± SE) of embryos of local goats of Assam

Stage of embryo	Diameter of embryos		Thickness of zona pellucida (μ)	Width of pervitelline space (μ)
	Including zona pellucida (μ)	Cell mass of embryo (μ)		
1-cell (4)	164.78±2.52	134.95±3.28	11.68±0.58	3.89±1.00
2-cell (4)	166.08±1.63	140.63±3.10	11.03±0.50	1.95±0.96
4-cell (4)	163.45±2.59	137.32±2.56	11.68±0.58	1.30±0.80
8-cell (4)	162.18±1.92	137.53±3.48	11.03±0.50	3.89±1.92
16-cell (4)	163.45±2.59	138.83±3.01	10.38±0.00	1.95±2.15
32-cell (4)	167.38±4.17	145.51±3.77	10.38±0.00	1.30±0.58
Early blastocyst (4) cyst (4)	162.19±1.92	144.02±1.00	10.38±0.00	0.00±0.00
Blasto cyst (4)	167.38±2.52	141.43±0.05	10.38±0.00	1.95±0.96
Late blasto-cyst (2)	173.87±1.56	153.11±1.56	10.38±0.00	0.00±0.00

SE = Standard error
Figures in parentheses indicate number of observations.

REFERENCES

Bonia, K.K. (1992) *A study on embryo transfer in Assam local goats*. PH.D. thesis, Assam Agricultural University, Guwahati-22.

Cole, H.H. and Cupps, P.T. (1977). *Reproduction in domestic animals* 3rd ed., Academic Press. New York, San Francisco, London. p. 286.

Hafez, E.S.E. (1987) *Reproduction in farm animals* . 5th ed., Lea and Febiger, Philadelphia, U.S.A. p.149.

Linares, T. and King, W.A. (1980) Morphological study of the bovine blastocyst with phase contrast mioroscopy. *Theriogenology, 64(2):* 125-133.

Mapletoff, R.J. (1986) *Bovine embryo transfer* . IN : Current therapy in Theriogenology, Vol. 2 (edt. Morrow, D.A.), Saunders Company, West Washington Swuare, PHiladelphia, P.A. 19195, pp. 54-58.

Salisbury, G.W.; Vandemark, N.L. and Lodge, J.R. (1985) *Physiology of Reproduction and Artificial Insemination of cattle* . 2nd ed., CBS Publishers and Distributor, 485, Jain Bhavan, Sahadra, Delhi-32, pp. 101-102.

Singh, R. and Sharmah, A. (1992) Morphological observation about goat oocytes aspirated from ovaries. Recent Advances in Goat Production : Proceedings of and paper presented at V International Conference on Goat, New Delhi, 2-5th March, pp. 1405-1408.

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A Study on *In-Vitro* Maturation of Oocyte in Cattle

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ABSTRACT

The cumulus-ocoyte-complexes (COC) were collected from the bovine ovary at abattoir using aspiration and dissection techniques from the antral folicles (1 to 5 mm diam). The number of oocytes recovered per ovary was 3.63±0.17 and 4.89±0.25 in aspiration and dissection techniques respectively. After processing, the COC were *in-vitro* cultured for 24 hours in m-KRB TCM-199 and Ham's F-12 culture media at 37° and 39°C.The incidence of Metaphase I was found to be highest (56.89%) in aspirated oocytas cultured in TCM-199 at 39°C. The incidence of Metaphase II was found to be the highest (37.09%) in dissected oocytes cultured in TCM- 199 at 39°C.

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The cumulus-cocyte-complexes (COC) liberated from their follicles by different techniques can spontaneously resume meiosis and complete the nuclear maturation when cultured (Shea *et al.,* 1976, Fulka *et al.,* 1982). The different incubation temperature and culture media were used for *in-vitro* culture of oocytes by various workers (Fukui *et al.,* 1982, Eng *et al.,* 1986). But, there is diversity of opinion regarding the best technique of recovery of oocytes, best media and incubation temperature for *in-vitro* maturation of oocyte. The present study was therefore undertaken to study the effect of two techniques of oocyte recovery, three culture media and two incubation temperatures on *in-vitro* maturation of oocyte.

MATERIALS AND METHODS

Bovine ovaries were collected from the private abattoir and brought to the laboratory in a thermosflask containing warm (37°C) physiological saline solution. The ovaries were washed thoroughly in tap water followed by warm physiological saline solution and Dulbecco's phosphate buffered saline (FES*). The cumulus oocyte complexes (COC) were collected from the ovarian follicles (of 2 to 5 mm diam) by aspiration and dissection techniques alternatively.

A total of 671 COC comprising 323 and 348 aspirated and dissected out oocytes were washed three times in PBS + 10% heat inactivated cow serum and two times in maturation medium (m-KRB. TCM- 199** and Ham's F-12***) under steriozoom microscope. The washed COC were then transfered to the maturation medium and incubated for 24 hours in a humidified atmosphere of approximately 5% CO_2 in air.

After incubation the *in-vitro* cultured oocyte was made nude and fixed in acetic alcohol (1:3) and stained with 1 per cent lacto-acetic lacmoid stain (Monaghan *et al.,* 1993). The status of *in-vitro* maturation was then examined at a magnification of 100x and 200x under a phasecontrast microscope.

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* No. TS 1006. HiMedia Lab. Pvt. Ltd., Bombay 400086, INDIA

** No. AT 094, Himedia Lab. Pvt. Ltd., Bombay 400086, INDIA

*** Nutrient Mixture F-12 (HAM) No. AT 085, Himedia Lab. Pvt. Ltd., Bombay 400086, INDIA

RESULTS AND DISCUSSION

The recovery of oocyte per ovary was found to be 3.63±0.17 and 4.89±0.25 in aspiration and dissection technique respectively the difference being highly significant (P>0.01).

Iwasaki *et al.,* (1967) and Berg and Brem (1989) recorded much higher number of oocytes per ovary in aspiration techniques. The discrepancy of recovery rates in different studies might be due to differences in the functional state of the ovaries in different ages, in different breeds, seasons and/or in the procedures of recovery. The significantly higher (P>0.01) recovery of oocytes in dissection technique might be due to less chance of missing the oocytes as the dissected follicles were ruptured under a steriozoom microscope during recovery of oocyte.

The rate of recovery of oocyte in left (4.33±0.24) and right (4.17±0.19) ovaries did not differ significantly. This is in agreement with the findings of Preinberg *et al.,*. (1989) and Carolan *et al.,* (1992).

The incidence of Metaphase I (56.89%) and Metaphase II (37.09%) was found to be highest in TCM- 199 cultured at 39°C in aspirated and dissected out oocytes respectively (Table). This finding was higher than the findings of Shea *et al.,*. (1976) but lower than that of Fukui *et al.,* (1982) and Eng *et al.,* (1986). The variation of findings in different studies might be due to the variation of pH of culture media (6.70 to 7.59), addition of gramulosa cells in the media (1-7 X 10^6/ml) prior to incubation and composition of media and conditions of incubation.

The effect of medium, incubation temperature and technique of recovery of oocyte on incidence of Metaphase I and Metaphase II was found to be non-significant. This is in agreement with the observations of Fulka *et al.,* (1982) and Katska and Smorag .(1985). The higher incidence of Metaphase I and Metaphase II at 39°C than at 37°C of incubation temperature might be due to the fact that *in-vitro* culture of oocyte at the core body temperature (39°C) of the species was advantageous than incubation at 37°C (Lens *et al.,*, 1983).

Table *in-vitro* maturation of bovine oocyte recovered using different techniques and cultured in different media and temperatures.

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medium/ Temperature	Metaphase-I (%)		Metaphase - II (%)	
	Aspiration	Dissection	Aspiration	Dissection
m-KRB				
37°C	43.47	39.62	30.43	26.42
39°C	47.82	54.05	28.99	33.78
TCM-199				
37°C	55.10	50.00	32.65	32.00
39°C	56.89	53.22	36.21	37.09
HF-12				
37°C	49.05	49.12	24.53	24.56
39°C	45.83	51.92	25.00	34.62

REFERENCES

Berg, U. and Brem, G. (1989) *Invitro* production of bovine blastooysts by *in-vitro* maturation and fertilization of oocytes and subsequent *in-vitro* culture. Zuchthygiene *24(3)* : 134-139 (Anim. Breed, Abstr. *58* : 2630).

Carolan, C., Monoghan, P., Mahmood, A., Lonargan, P., Gollagher, M. and Gordon, J. (1992) Slicing of bovine ovaries as a means of oocyte recovery.J. Reprod. Fert. Abstr. Series, *9* : 88.

Eng, L. A., Kornegay, E.T., Huntington, J. and Wellman, T. (1986) Effects of incubation temperature and bicarbonate on maturation of pig oocytes *in-vitro*. J. Reprod. Fert. *76* (2): 657-662 (Anim. Breed. Abstr. *54* : 3921).

Fukui, Y., Fukushima, M., Teravaki, Y. and Ono, H. (1982) Effects of gonadotropins, steroids and culture media on bovine oocyte maturation *in-vitro*. Theriogenology, *18* (2) : 161-175.

Fulka, J. (Jr.), Pavlok, A. and Fulka, J. (1982) *In-vitro* fertilization of sona-free bovine occyte matured in culture. J. Reprod. Fert. *64* : 495-499.

Iwasaki, S.: Kono, T., Nakahara, T., Shioya, T., Fukushima, M. and Hanada, A. (1987) New methods for the recovery of oocytes from bovine ovarian tissue in relation to *in-vitro* maturation and fertilization. Japanese J. Anim, Reprod. *33* : 188-192.

Katska, L. and Snorag, L. (1985) The influence of culture temperature on *in-vitro* maturation of bovine cocytos. Anim. Reprod. Sci. *9* (3): 205-212 (Anim. Breed, Abstr. *54* : 854).

Lens, R.W., Ball, G.D., Leibfried, M.L., Ax: R.L. and First, N.L. (1983) *in-vitro* maturation and fertilization of bovine occytes are temperature depandent process. Biol. Reprod. *29* : 173-179.

Monaghan, P.: Cerolan, C.: Lonergan, P., Sharif, H., Wahid, H. and Gordon, I. (1993) The effect of maturation time on subsequent *in-vitro* development of bovine oocytes. Theriogenology. *39*: 270.

Prelnberg, G.A., Tirmanis, I. Ya. and Turka, L.K. (1989) Quantitative and qualitative evaluation of isolated oocytes in cows. Referativnyi Zhurnal, *58* (2): 349 (Anim. Breed. Abstr. *58* 3533)

Shea, B.F., Latour, J.P.A., Bedirian, K.M. and Baker, R.D. (1976) Maturation *invitro* and subsequent penetrabilityof bovine follicular oocytes. J. Anim. Sci. *43* (4): 809-815.

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SDS-Polyacrylamide Gel Electrophoresis of Proteins of Spermatozoa from Cauda Epididymis, Vas deferens and Ejaculated Spermatozoa of Buffalo Bulls.

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ABSTRACT

Changes in protein profiles of whole spermatozoa from cauda epididymis vas deferens and ejaculated spermatozoa of buffalo bulls were studied by SDS-PAGE analysis. Distinct changes were observed in the protein profiles of spermatozoa from cauda epididymis, vas deferens and ejaculated spermatozoa. Twelve, 11 and 23 protein bands of molecular weight ranging from 93-94 to 11 KDa were observed in spermatozoa from the cauda epididymis, vas deferens and ejaculated spermatozoa respectively.

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The epididymal transit of spermatozoa is characterized by complex biochemical and physiological changes in the spermatozoa (Kaur *et al.,* 1991; Boue and Sullivan, 1993; Moore *et al.,*. 1994). These changes are related with the maturation of spermatozoa, development of forward motility, viability and fertility of the spermatozoa. The biological role of epididymis in the maturation of spermatozoa in various mammalian species has been reviewed (Amann *et al.,* 1993). The changes in the surface proteins of the bull spermatozoa during epididymal maturation (Vierulla and Rajaniemi, 1981) have been reported. The present study was taken up to investigate the changes in protein profiles of the buffalo bull spermatozoa during epididymal and vas deferens transit in comparison with ejaculated spermatozoa by SDS-PAGE analysis.

MATERIALS AND METHODS

Twelve pairs of testes with intact tunica albugenia along with spermatic cord, epididymis and vas deferens were collected from healthy buffalo bulls immediately after slaughter at a local abattoir, transported to the laboratory in ice, washed and the epididymis and vas deferens were dissected for collection of spermatozoa.

The details of collection of spermatozoa from the cauda epididymis, and vas deferens and semen samples from normospermic, fertile buffalo bulls, separation of spermatozoa, their washing and ultrasonication have been reported earlier (Kulkarni *et al.,* 1997; 1998).

SDS-PAGE analysis of whole sperm proteins from the cauda epididymis, vas deferens and ejaculated spermatozoa was done according to Laemmli (1970) as reported earlier (Kulkarni *et al.,* 1997).

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RESULTS AND DISCUSSION

Representative SDS-PAGE patterns of proteins of spermatozoa from cauda epididymis, vas deferens and ejaculated spermatozoa of buffalo bulls are presented in Fig. It is evident that the spermatozoa from three different sources showed distinct changes in protein patterns on SDS-PAGE analysis. Twelve, 11 and 23 protein bands of mol. wt. ranging from 93-94 to 11 KDa were observed in whole spermatozoa from cauda epididymis, vas deferens and ejaculated spermatozoa respectively. Proteins of mol. wt. 82, 58, 52, 45, 43, 39, 36, 34-35, 28, 23, 19-20, 18, 16 and 14 KDa were present in ejaculated spermatozoa and absent in spermatozoa from other two sources. Proteins of mol. wt. 93-94, 72-74, 48, 47, 40, 30-32, 24-25 and 17 KDs were present in all the three sperm populations. A protein of mol. wt. 30-32 KDa was the major protein in the cauda epididymal, vas deferens and ejaculated sparmatozoa.

The results of our study indicate distinct changes in protein patterns of whole spermatozoa from cauda epididymis, vas deferens and ejaculated spermatozoa of buffalo bulls. These changes are in accord with those reported in the rat (Kaur *et al.,* 1991) and chimpanzee (Young *et al.,* 1985). In the golden hamster the maturational changes in the spermatozoa during epididymal transit showed significant increase in the progressive motility in the cauda epididymal sparmatozoa as compared with the sparmatozoa from the caput and corpus epididymis (Weissenberg *et al.,* 1994).

In the human, hamster and rat a cauda epididymal sperm protein with mol. wt. 26 KDa was found to be implicated in fertilization. This protein was synthesised and secreted by the epididymal epithelium and was adsorbed on spermatozoa during epididymal maturation (Boue and Sullivan, 1993; Moore *et al.,* 1994).

The increased number of protein bands in the ejaculated spermatozoa (23) as compared with the spermatozoa of cauda epididymis (12) and vas deferens (11) observed in the present study, could be due to adsorption of proteins on the surface of the spermatozoa after ejaculation. In cattle bulls the major proteins of seminal plasma with mol. wt. 15, 16.5, and 30 KDa originated from seminal vesicles were reported to be adsorbed on the spermatozoa after ejaculation (Desnoyers *et al.,* 1994).

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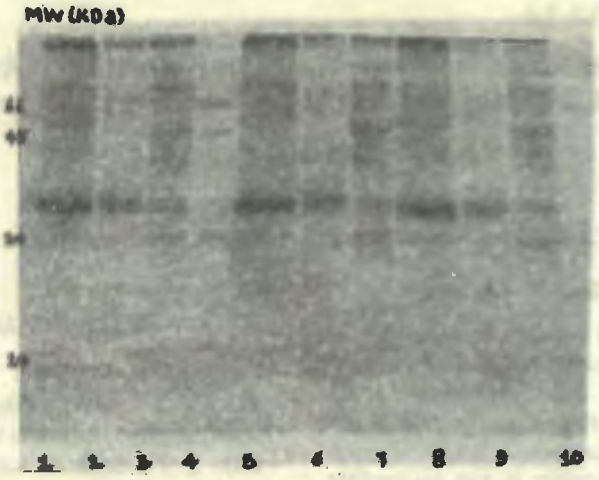

SDS-PAGE Patterns of sperm proteins from Cauda epididymis, Vas deferens and ejaculated spermatozoa of buffalo bulls. Lane, 1, 5, 8 Cauda epididymis sperms, 2, 6, 9, vas deferens sperms 3, 7, 10 ejaculated spermatozoa, 4, molecular weight markers.

REFERENCES

Amann, R.P., Hammerstedt, R.H. and Veermachaneni, D.H.R. 1993. The epididymis and sperm maturation. Reproduction and Fertility Development *5* : 361-381.

Boue. F. and Sullivan, R. 1993. Human epididymal sperm Protein involved in fertilization. Biology of Reproduction *48:* Suppl I P. 85. Abatr. 198.

Desnoyers, L. Therien, J.and Manjunath, P. 1994. Characterization of the major proteins of bovine seminal plasma by two Dimensional polyacrylamide gel electrophoresis. Molecular Reproduction and Development *37:* 425-435.

Kaur, J. Ramkrishnan, P.R. and Rajalakshmi, M. 1991. Alteration of protein profiles of rat spermatozoa during maturation. Andrologia *23:* 53-56.

Kulkarni, B.A. Rupal, R.K. and Hegde, U.C. 1997. SDS-polyacrylamide gel electrophoresis of sperm proteins of buffalo and cattle bulls. Indian Journal of Animal Sciences. *67:*110-112.

Kulkarni, B.A., Neena P. Suratkar and U.C. Hegde (1998). SDS-polyacrylamide gel electrophoresis of proteins of cauda epididymal fluid, vas deferens fluid and seminal plasma of buffalo bulls. Buffalo Journal (In press).

Laemmli, V.K. 1970. Cleavage of structural proteins during assembly of Bacteriophage T4. Nature (London) *227:* 680-685.

Moore, A., Ensurd, K.M., White, T.W., Frethem, C.D. and Hamilton, D.W. 1994. Rat epididymis specific sperm maturation antigens. I. Evidence that the 26 KDa 4E9 Antigen found on rat caudal epididymal sperm tail is derived from a protein secreted by the epididymis. Molecular Reproduction and Development *37:* 1810194.

Vierula, M. and Rajaniami, H. 1981. Changes in surface protein structure of bull spermatozoa during epididymal maturation. Internatinal Journal of Andrology. *4:* 314-320.

Weissenberg, R. Yossefi, S. Oschry, Y.M. and Levin, L.M. 1994. Investigation of epididymal sperm maturation in the golden Hamster. International Journal of Andrology. *17:* 256-261.

Young, L.G., Hinton, B.T. and Gould, K.G. 1985. Surface changes in chimpanzee sperm during epididymal transit. Biology of Reproduction. *32:* 399-412.

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Note on Planimetric Estimation of Surface Area of Microscopic Object of Definite but Irregular Geometric Shape.

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ABSTRACT

Bovine sperm head has definite but irregular geometric shape. For determination of its area with camera lucida and planimeter it is incumbment to assess area magnification at the optical center of the projected image of each particle. Linear magnification is not equipollent to area magnification. Area magnification of the projected object alters with alteration of axis alignment of the object in the optical field of the microscope.

———x———x———

Estimation of surface area of sperm head is important in relation to the study of hydrodynamics of movement as also for calculating energy cost of swimming sperm in the fluid matrix. Clossest approximation of the area of surface of irregular boundaries of microscopic objects like sperm head are generally made either by pianimeter or graphic integration of micrograph. A more accurate method of ascertaining the surface area of the cell has been described by Glick (1961) by using a planimeter attached with the microscope. In planimetric measurements most workers (Deb *et al.*, 1964, Pant and Mukherjee 1973) use linear magnification instead of area magnification of the projected image for the purpose of calculation of true area of the microscopic object. This leads to an inherent error in the method used by them as linear magnification cannot be equated with area magnification. The present note analyses the difference in linear and area magnification of projected image of the same microscopic object, sperm head, for correct estimation of the area of sperm head.

MATERIALS AND METHODS

Semen samples from various breeds of bulls used routinely for artificial insemination purpose were collected, diluted with 2.9% sodium citrate dihydrate buffer solution in the proportion of 1:3 and activated at 35°C. One drop of this diluted semen sample and 1 drop of 1% aquous citrate buffer mixed on a glass slide by air blowing through a pipette. To this one drop of 4% aniline blue solution in citrate buffer was added again mixed by air blowing and kept for 30 seconds. A fine film was then drawn and air dried. The film was then fixed in 5% formalin vapour at refrigerated temprature overnight. Unstained slides were also prepared.

Slides were examined under phase contrast microscope. A calibrated ocular micrometer was placed in the eye piece of the microscope. Linear mensurations of sperm head were done at 1000 X magnification. Two linear dimensions, length and maximum breadth of each sperm head were measured.

Distance between the height of the convexity at the base, the implantation fossa, to that at the apex along longitudinal axis was taken as the length (L) of the head. Maximum breadth (Bmax) of the head of spermatozoa was measured by rotating the occular micrometer and holding the scale parallel to the base line of the spermatozoa near its apical end at the broadest part. To measure the Bmax, when necessary, the slide was moved laterally and or

antero-posteriorely. The centre of the head of the cell selected for measurement was fixed at an indicator point. The indicator point was fixed in the eye piece.

The halo effect of the phase contrast microscope was nullfiied by focussing microscope at a point when the membrane boundary was noted as a clear sharp line and no discernable optical distortion occured. All cells measured were having their longitudinal axis parallel to X-axis of the optical field and all the sperms were lying completely flat on the flat surface of its head. Sperm heads with angular elevations were not considered for measurement.

To examine whether the magnification altered with alteration of position the same sperm head was projected at four different angles by rotating the stage of the microscope through an angle of approximately 45° at each step from the horizontal plane. Thus the images were accordingly projected at four different angular position of the major axis of sperm head,

Spermatozoa head lying flat on slide was projected on a white paper lying on the table by the side of the microscope with the help of camera lucida attached on the eye piece of the microscope. The cells for measurements were fixed at a fixed point and pencil tracing of the outline of the projected images were taken on a white art paper. The area of the traced image was then found out with the help of a planimeter. Average of four readings of the area of each projected image was taken for record and further calculation. The linear measurements of the image outline were taken with a dial type vernier calliper capable of reading upto second decimal point in mm scale.

The area of the rectangle containing the sperm head (a1) was determined by multiplication of length (L) and maximum breadth (B_{max}) obtained from occular micrometer.The area of the rectangle containing the projected image of the same spermatozoa head (a2) was obtained by multiplication of L and B_{max} of the projected image. The area magnification at the centre of the optical section of the projected image is obtained by dividing a^2 by a1. This method of calculation of magnification nullifies the inherent error in the usual practice of accepting linear magnification of the projected image as the area magnification.

RESULTS AND DISCUSSION

The flat head of spermatozoa is lodged within a rectangle formed by the length and breadth at the broadest part of the sperm head. Truly the spermatozoa head area is smaller than the area of the rectangle. To find out the real area of the sperm head a part of the rectangle has got to be deducted. As sperm head is bound by curves conforming more closely to a regular standard type (Van Duijn, 1960) the area to be deducted shall be a constant portion of the rectangle formed by length and breadth at the widest part of the sperm head, or in other words, the area of the sperm head itself is a constant proportion of the rectangle. As such the magnification at the centre of the head area of the projected image can be assumed as same to that of the rectangle formed by L and B_{max} of the sperm head.

During calculation of magnification, linear and area, of projected image it is noted that the magnification varied widely though the sperm head was placed on the fixed indicator point and the tracing paper was kept at the same distance from camera lucida. The variation may be in the first place

due to alteration in the angle of incidence and reflection of the optical rays and or to an alteration in the distance of the projected image from camera lucida.

Coefficient of variation of the magnification of each projected sperm head through four different positions are calculated. Finally mean ± standard deviation of coefficient of variations of all cells was calculated. The final values were 13.4±2 75, 11.14±3.39 and 6.62±3.81 for length, breadth and area respectively. It has been noted that the dispersion in terms of coefficient of variation at four different axial positions of the same sperm head on the said fixed indicator point were 20.52%, 29.74% and 57.55% for length, breadth and area respectively. Similarly it is also noted that the final mean ±s.d. of coefficient of variation of planimeter readings of the projected area of the same sperm heads came to 6.82±4.32 and thus finally the dispersion of the mean area in terms of coefficient of variation of the projected image was 63.34%. These values of dispersion are quite high specially with respect to area magnification. This is reflected in the dispersion of planimeter readings of the projected head area at the four axial position and thus influences the real value to an extent which makes the result unreliable. This suggests that rotation or differences in the angle of placement of the axis of sperm head could alter the magnification and thus alter the area of the projected image itself. The result establishes that the magnification significantly ($P \leq 0.01$) altered with alteration of axial position of the sperm head under the microscope.

Finally it is suggested that for measurement of area of microscopic objects geometric conformation of the particle should be carefully noted. It if follows a regular geometric shape and pattern the specific formula of geometry should be used for estimation of surface area of such particle. For particle with definite but irregular geometric shape only planimetric method be used. In planimetric method area magnification at the centre of the particle has got to be obtained to avoid any error because of alterationin position and / or axis of the particle.

REFERENCES

Dab C., Boral M.C. and Sarkar C. (1964). Measurement of hepatic parenchymal cells and nuclear volume in different classes of vartebrates. Anatomical Record *148:* 499-501.

Glick D. (1961). quantitative chemical technique of histochemistry and cytochemistry. Vol. I. Interscience Publishers, New York, USA.

Pant K.P. and Mukherjee, D.P. (1973). Breed differences in characteristics of buffalo spermatozoa. Ind. J. Dairy Sc. *26:* 183-187.

Van Duijn C. Jar. (1960). Mensuration of the heads of bull spermatozoa. Microskopie Bd. *14:* 265-274.

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Seasonal Influence on the Quality and Freezability of Semen of Friesian and Murrah Buffalo bulls*

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ABSTRACT

Semen ejaculates of 3 Friesian and 3 Murrah bulls were studied biweekly over a period of 1 year (hot, humid and cold seasons) to know the seasonal influence on its quality and freezability. Overall mean semen picture in Friesian and Murrah bulls was: ejaculate volume 5.12±0.18 and 3.99±0.13 ml; mass activity score 3.43±0.12 and 3.48±0.19; sperm count / ml 949.17±28.39 and 1264.86±34.83 million; Live sperm 87.35±0.54 and 88.07±0.59%; abnormal sperm 9.26±0.43 and 9.28±0.42%, and freezability (post-thaw motility) 50.22±1.23 and 47.26±1.59% respectively. The values of sperm motility, viability, concentration and freezability were significantly ($P<0.05$) lower and volume plus abnormal sperm were higher during cold season (Nov-Feb) in Murrahs. In Friesians also, the mass activity and sperm concentration were significantly lower with higher ejaculate volume during that season. Semen ejaculates of excellent quality and freezability were donated by bulls of both the species during the months of March-April and Sept-October, and of poor or nonmotile type during the months of Dec-January when the ambient temperature was extremely low.

———x———x———

Both the quality and freezability, and even fertility of bovine semen has been reported to vary remarkably during the extremes of summer and winter seasons (Siddhu and Guraya, 1979; Hosmani and Basavaiah, 1986; Dhami *et al.,* 1987; Khokhar *et al.,* 1987). This is particularly so in the northern states of our country (Gill *et al.,* 1974; Bhattacharya *et al.,* 1978; Mudgal and Radhe Syam, 1985). The present study was aimed to ascertain the best season / months for harvesting and freezing maximum number of good quality ejaculates of Friesian and Murrah bulls to achieve better fertility results in the field.

MATERIALS AND METHODS

This study was undertaken over a period of 1 year (1990-91) utilizing biweekly collected semen ejaculates of 3 Friesian and 3 Murrah bulls of IVRI, Izatnagar (UP). The year was divided into hot (Mar-June), humid (Jul-Oct) and cold (Nov-Feb) seasons as per the prevailing climatic conditions. The bulls were fed and managed identically round the year and were under weekly semen collection schedule in AV. Immediately after collection, each ejaculate was evaluated macro- and microscopically. The samples having more than 70% initial motility were diluted in standard Tris diluent, keeping 50 million sperm / ml, filled in 0.5 ml straws and frozen in liquid nitrogen vapour (Dhami *et al.,* 1987). Freezability was assessed by thawing frozen semen straws in water-bath kept at 40°C for 30 sec and examining the percentage of progressively motile

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sperm under high power phase contrast microscope fitted with a biotherm stage. The data were analysed statistically and seasonal means were compared by Duncun's new multiple range test.

RESULTS AND DISCUSSION

The averages of some important seminal attributes and freezability of semen of Friesian and Murrah bulls observed during different seasons of the year have been presented in Table 1. The findings revealed that the sperm concentration was significantly ($P<0.05$)higher in Murrahs and the volume plus freezability in Friesians. The means of remaining traits did not differ between them. These findings for species difference and the values of most traits compared well with the reports of Saxena and Tripathi (1979) and Khokhar *et al.,* (1987). Although they found sperm concentration to be significantly lower in Murrahs than the taurine bulls. Dube *et al.,* (1982), however, reported significantly higher motility and sperm count in Murrahs than in Jersey bulls. The consistently low sperm count / ml observed in Friesian bulls as compared to Murrahs in our study was attributed to poor adaptability of taurine bulls in the Tarai climate (Saxena and Tripathi, 1979; Dhami and Sahni, 1994). Further, significantly higher freezability found for semen of Friesians as compared to Murrahs also coincided well with the previous report of Mohan *et al.,* (1992). Overall, the mean semen picture and the freezability observed were within the reported normal range for both the species.

The effect of seasons was found to be significant ($P<0.05$) for ejaculate volume, mass activity, sperm concentration per ml and per ejaculate and freezability in both the species, and also for individual motility, live sperm and abnormal sperm percent in Murrahs. Density score, however, did not show any seasonal variation in either of the species. These findings especially for buffalo semen are in agreement with the reports of Gill *et al.,* (1974). Hosmani and Basavaiah (1986) and Dhami *et al.,* (1987). Similar seasonal variations in the quality and freezability of ox semen has also been reported from abroad (Ali *et al.,* (1981) and in India (Khokhar *et al.,* 1987).

During cold seasons, the ejaculate volume was observed to be significantly ($P<0.05$) higher and the mass activity, sperm concentration / ml and freezability were lower in both the species. The values of individual sperm motility and live sperm percent were also significantly lower with higher abnormal sperm percent during that season in Murrahs. Although the differences between hot and humid seasons were not significant for any of the seminal traits studied in Friesian and Murrah bulls (Table 1) the inferior quality and low freezability of semen observed in both the species during cold season was attributed to extremely low ambient temperature and chilly winds that prevailed during the months of December-January. Gill *et al.,* (1974) also reported poor quality semen between November and February months.The too low temperature and chilly winds adversely affect the vigour and testosterone production of bulls thereby suppressing the testicular function and acoessory sex glands secretion as opined by Mudgal and Radhe Syam (1985). This view was also supported by high frequency of nonmotile / static ejaculates obtained during those two months in both the species (Dhami and Sahni, 1994). The high humidity prevailed in the months of July-August also temporarily suppressed the performance and quality of semen especially in Murrahs. On the other hand, the hot dry atmosphere prevailed during the months of March-April and again

in September-October favoured production of excellent quality semen with high freezability in both the species. Siddhu and Guraya (1979) reported the semen quality of Murrahs to be the best in Spring and Summer and worst in the rainy season.

The present findings clearly suggested that Murrah and Friesian bulls exhibited identical influence of seasons on their reproductive performance and semen freezability in the Tarai region of Uttar Pradesh, and there exhisted a positive association between initial semen quality and freezability and even fertility (Dhami *et al.*, 1987). Therefore, to obtain higher conception rates using frozen semen maximum number of good to excellent quality ejaculates should be frozen during favourable months of the year from the sires of choice of both the species.

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Table 1. Seasonal variations in the quality and freezability of semen of Friesian and Murrah bulls

Bulls / Seminal attributes	Seasons			Pooled
	Hot (Mar-Jun)	Humid (Jul-Oct)	Cold (Nov-Feb)	(Jan-Dec)
Friesian bulls:				
Ejaculate volume (ml)	5.20 ±0.41^{a}	4.68 ±0.97^{b}	5.49 ±0.19^{a}	5.12 ±0.18
Density score (0-3)	2.57 ±0.17	2.74 ±0.12	2.48 ±0.15	2.60 ±0.08
Mass activity score (0-5)	3.61 ±0.18^{a}	3.56 ±0.23^{a}	3.13 ±0.12^{b}	3.43 ±0.12
Individual motility (%)	84.44 ±0.98	83.33 ±0.99	83.52 ±0.79	83.80 ±0.78
Sperm count / ml (million)	974.44 ±69.72^{a}	970.56 ±37.28^{a}	902.50 ±37.43^{a}	949.17 ±28.39
Sperm count / ejac. (million)	4836.39 ±316.93^{ab}	4506.44 ±268.92^{b}	4964.69 ±232.48^{a}	4768.84 ±131.67
Live sperm (%)	87.44 ±0.71	87.78 ±0.72	86.83 ±0.48	87.35 ±0.54
Abnormal sperm (%)	9.94 ±0.63	9.00 ±0.52	8.86 ±0.38	9.26 ±0.33
Freezability (%)	52.71 ±1.64^{a}	48.91 ±2.28^{ab}	47.87 ±1.82^{b}	50.22 ±1.23
Murrah bulls:				
Ejaculate volume (ml)	3.70 ±0.20^{b}	3.66 ±0.29^{b}	4.61 ±0.16^{b}	3.99 ±0.13*
Density Score (0-3)	2.63 ±0.10	2.65 ±0.09	2.57 ±0.13	2.62 ±0.06

Mass activity score (0-5)	3.89 ±0.22[a]	3.92 ±0.17[a]	2.62 ±0.28[b]	3.48 ±0.19
Individual motility (%)	87.50 ±1.09	85.56 ±1.27[a]	82.78 ±1.90[b]	85.28 ±1.07
Sperm count / ml (million)	1262.21 ±93.88[a]	1365.15 ±120.23[a]	1167.22 ±64.79[b]	1264.86 ±34.83*
Sperm count / ejac. (million)	4681.56 ±192.05[b]	4996.45 ±323.12[ab]	5371.39 ±232.24[a]	4983.14 ±170.88
Live sperm (%)	89.39 ±0.98[a]	89.22 ±0.83[a]	85.61 ±0.53[b]	88.07 ±0.59
Abnormal sperm (%)	8.33 ±0.70[b]	7.83 ±0.57[b]	11.64 ±0.62[a]	9.28 ±0.42
Freezability (%)	50.56 ±2.13[a]	47.04 ±2.48[ab]	44.17 ±2.16[b]	47.26 ±1.59*

Means bearing superscript in-common do not differ significantly between seasons within the breed group.

* ($P<0.05$) between species / breeds.

REFERENCES

Ali, H.M., Ahmad, I.A. and Yassen, A.M. (1981). Sexual development and post-pubertal changes in seminal characteristics of buffalo and Friesian bulls. *Alexandria J. AGri. Res., 29:47* (ABA *52:* 5695).

Bhattacharya, M.K., King, K.G. and Batra, T.N. (1978). Buffalo semen quality in various seasons. *Indian Vet. J., 55:* 591094.

Dhami, A.J., Jani, V.R. and Kodagali, S.B. (1987). Fertilityof frozen semen in Surti buffaloes. *Current Science, 56:* 1193-94.

Dhami, A.J. and Sahni, K.L. (1994). Comparative appraisal of physicomorphological and enzymatic attributes of semen and their interrelation ships in ox and buffalo bulls. *J. Appl. Ani. Res., 5:* 13-20.

Dube, G.D., Pareek, P;K., Dwarakanath, P.K. and Vyas, K.K. (1982). Lactic dehydrogenase in relation to semen quality. *Ind. J. Dairy Sci., et:* 80.82.

Gill, R.S., Gangwar, P.G. and Takkar, O.P. (1974). Seminal Attributes in buffalo bulls as affected by seasons. *Indian J. Ani. Sci. 44:* 415.18.

Hosmani, S.V. and Basavaiah, P. (1986). Effects of seasonal variations on frozen semen production of Surti buffalo bulls. *IJAR, 7(2):* 22-25.

Khokhar, B.S., Singh, M. and Chaudhary, K.C. (1987). Transaminases in cattle and buffalo semen in relation to fertility and seminal characteristics during moderate and cooler seasons.*Anim. Reprod. Sci., 13:* 177-82.

Moha, G., Sahni, K.L., Dhami, A.J. and Tripathi, R.P. (1992). Prediction of freezability on the basis of seminal enzymatic profiles in Murrah, Friesian and cossbred bulls. *Indian J. Anim. Sci., 62:* 111-115.

Mudgal, V.D. and Radhe Syam (1985). Seasonal influences on semen quality of buffalo bulls. *All India Symp Seminology. Pantnagar,* Oct 29-30.

Saxena, V.B. and Tripathi, S.S. (1979). Seminal characteristics and preservability in Red Dane bulls. *Indian J. Anim.Sci., 49:* 878-83.

Siddhu, K.S. and Guraya, S.S. (1979). Effect of seasons on physiochemical characteristics of buffalo semen. *Indian J. Anim. Sci., 49:* 884-89.

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Influence of Programmable Freezing on Cryosurvival of Awassi Ram Spermatozoa

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ABSTRACT

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 spermatizoa 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, curvilineer velocity, amplitude of lateral head displacement and beat frequency of spermatozoa.

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The Awassi is a predominant and widespread type of sheep in South West Asia (Epstein, 1977). The "triple purpose" productive attributes of this breed for milk, meat and wool are novel under the vast sub-tropical environment in the semi-arid or arid regions (Epstein, 1982). Cryopereservation 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.

MATERIALS AND METHODS

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 spermatozoa (0-4scale). Ejaculates having thick consistency, rapid wave motion (+5), spermatozoa concentration < 300) million per ml, 90% initial motility with +4 rating of intensity of movement were pooled and diluted @ 1000 million spermatozoa per ml (Mathur and Joshi, 1996) using egg yolk tris glycerol extender (Schmehl *et al.,* 1986).

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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 -120°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).

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 μ m/sec, % linearity, % straightness, amplitude of lateral head displacement (ALH, μ m) and beat frequency (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 arc 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 initial 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 spermatozoa 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 spermatozoa 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 spermatozoa.

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 (μm/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 (μ m) 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 percent 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 spermatozoa could be achieved by programmable freezing and computerized assessment.

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REFERENCES

Aitken, R.J. (199). Motility parameters and fertility. Controls of sperm motility: Biological and clinical aspects.pp. 285-302. (Ed.) Gagnon C. CRC Press, Boca Raton.

Budworth, P.R.; Amann, R.P. and Chapman, P.L. (1988). Relationships between computerized measurements of motion of frozen thawed bull spermatozoa and fertility. Journal of Andrology, *9:* 41-53.

Epstein, H. (1977). The Awassi sheep in Israel. World Review of Animal Production, *13(2):* 19-26.

Epstein, H (1982) Awassi Sheep, World Animal Review, *44:* 9-18.

Ipsen, J. and Feigl, P. (1970), Bancroft's Introduction to Biostatistics.2nd Edition, Harper and Row Publishers, New York, pp 55-64.

Joshi, Anil and Mathur, A.K. (1994). Influence of freezing rate on post-thaw motility and velocity of ram spermatozoa. Proceeding of 7th International Symposium on Spermatology, pp 9. 3-9.4,9-14 October, CAirns, Australia.

Joshi, Anil and Mathur, A.K. (1996). Assessment of sperm kinematics of frozen-thawed ram spermatozoa. Indian Journal of Animal Reproduction, *17:* 42.44.

Mathur, A.K. and Joshi, Anil (1994). Effect of cryopreservation on acrosomal integrity and viability of ram spermatozoa during incubation. Proceeding of the 7th International Symposium on Spermatology, pp 9.7-9.8, 9-14 October, Cairn,s Australia.

Mathur, A.K. and Joshi, Anil (1996). Effect of cryopreservation on acrosomal integrity and viability of ram spermatozoa during incubation. Proceeding of the 7th International Symposium on Spermatology. pp 9.7-9.8, 9-14 October, Cairn,s Australia.

Mathur, A.K. and Joshi, Anil (1996). Programmable freezing and computerized assessment of ram spermatozoa. Indian Journal of Animal Sciences, *66:;* 251-252.

Parkinson, T.J. and Whitfield. C.H. (1987). Optimisation of freezing conditions for bovine spermatozoa. Theriogenology, *27:* 781-797.

Pontbraind, D.; Howard; J.G.; Schiewe, M.C.; Stuart, L.D. and Wildt. D.E. (1989). Effect of cryoprotective diluent and method of freeze-thawing on survival and acrosomal integrity of ram spermatozoa. Cryobiooogy, *26:* 341-354.

Salamon, S. and Maxwell, W.M.C. (1995). Frozen storage of ram semen 1: Processing, freezing, thawing and fertility after cervical insemination. Animal Reproduction Sciences, *37:* 185-249.

Schmehl, M.K.; Vazques, I.A. and Grahm, E.F. (1986). The effect of dialysis of extended ram semen prior to freezing on post-thaw survival and fertility. Cryobiology, *23:* 406-414.

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Studies on sexual behaviour and seminal quality characteristics of Surti buffalo bulls and their interrelationships.

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ABSTRACT

Sexual behaviour and semen quality was studied in four Surti buffalo bulls for a period of four months. The overall mean values of libido score, service behaviour, reaction time, flehmen response (percent - per ejaculate), dismounting time, munting stimulus (percent per ejaculate) were 76.53±3.12%, 62.24±2.12%, 108.6±6.20 seconds, 87.75%, 5.63±0.36 seconds and 59.18% respectively. The overall mean values of semen quality were semen volume 2.22±0.11ml, mass activity +3.48±0.19, sperm motility 72.45±2.22%, sperm concentration 941.02 ±28.32 $x10^6$ sperms / ml and percent live sperms 77.04 ±2.08 respectively. The analysis of variance showed that the semen quality characteristics did not differ between the bulls and between the months studied. Libido score was significantly positively correlated ($P<0.5$) with semen volume, all other correlations between sexual behaviour and semen quality parameters were not significant. Semen volume was also negatively correlated with other semen quality characteristics.

———x———x———

Medium sized buffaloes have remained the mainstay for many farmers in some parts of the country. Seminal quality and sexual behaviour do show some interrelationships which has been studied for the medium sized buffalo bulls and results are presented in this paper.

MATERIALS AND METHODS

Four Surti buffalo bulls aged between 3 to 4 years and belonging to the AICRP on Buffaloes, Livestock Research Station Vallabhnagar maintained under identical conditions were utilized for the present study. The libido score and service behaviour of bulls was recorded on the score card at the time of semen collection as described by Singh and Panganwkar (1989). The reaction time and the time taken by the bull in dismounting was recorded by a stop watch. The semen of the bulls was collected by using artificial vagina and the various sexual activities recorded during collection. The ejaculates were evaluated for semen quality and physical characteristics by standard procedures of Hermann and Madden (1953). The values of different semen quality characteristics were evaluated between the bulls and between the months by analysis of variance. The parameters of semen quality and sexual behaviour were analyzed for existence of correlation between them as per standard statistical procedures of Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The overall mean sexual behaviour and seminal quality characteristics of Surti buffalo bulls are presented in Table 1 which is self-explanatory. Many parameters closely resemble earlier works of Dhami and Kodagali (1988), Sharma *et al.*, (1994) and others, but vary from a few reported findings of Manoj Kumar and Nagpaul (1995) and others. The analysis of variance for seminal quality characteristics showed nonsignificant differences for comparison between the 4 buffalo bulls and between

the four months studied (July-October) indicating that neither the months nor the individual bull had any effect on the semen quality of Surti buffalo bulls. This finding closely corroborate with those of Hosmani and Basavaiah (1986). Results of analysis of coefficient of correlation between the sexual behaviour parameters and semen quality characteristics are presented in Table 2. All the positive and negative correlations observed were statistically non-significant except the correlation between libido score and semen volume which was significant ($P<0.05$, t value 2.728). Amongst the semen quality characters a negative correlation between semen volume and other semen characters was seen. This is in agreement with findings of Dhami and Kodagali (1988) in Surti buffalo bulls.

Table 1. Overallm mean sexual behaviour and semen quality charachteristics of Surti buffalo bulls.

Sexual Behaviour	Mean Value	Semen Quality	Mean Value
Libido Score %	**76.53±3.12**	Semen Volume (ml)	2.22±0.11
Reaction Time Sec.	108.6±6.20	Mass activity	3.48±0.19
Service behaviour%	62.24±2.12	Sperm motility%	72.45±2.22
Dismounting time Sec	5.63±0.36	Sperm Conc.(10/ml)	941.02±28
Flehmen Response %	87.75	% Live sperms	77.04±2.0
Mounting stimulus required	59.18%		

Table 2: Correlation Table (r values)

Coeficient of Correlation	Semen Volume	Mass Activity	Sperm Motility	Sperm Conc.	%Live Sperms
Libido Score	0.37	0.12	0.10	0.13	0.18
Service Behaviour	-0.025	-0.084	-0.064	-0.015	0.030
Reaction Time	-0.221	0.043	0.010	-0.117	-0.068

REFERENCES

Dhami, A.J. and Kodagali, S.B. (1988) Seminal charachteristics and their interrelationships in Surti buffalo. Indian Vet. J. 65 *(1):* 61-64.

Hafez, E.S.E (1987): Reproduction in farm animals. Lea & Febriger, Philadelphia, U.S.A.

Hermann, H.A. and Madden, E.W. (1953) The artificial insemination of dairy cattle. A Hand book laboratory manual. Lucas brothers, Columbia, Missourri. U.S.A.

Hosmani, S.V. and Basavaiah, P. (1986) Effect of seasonal variations on frozen semen production of surti buffalo bulls. Indian J. Ani. Reprod., *7(2):* 22-25.

Manojkumar and Nagpaul, P.K. (1995). Assessment of sexual behaviour in Sahiwal and Murrah bull. Indan J. Anim. Reprod. Magt., *11(4):* 217-221.

Sharma, R. K., Pangaonker, G.R., Verma, H.K. and Mehar Singh (1994) Physico--biochemical practice of semen in relation to libido and service behaviour in buffalo bull. Indian J. Anim. Reprod., *15(1):* 60-62.

Snedecor, G.W. and Cochran, W.G. (1967) Statistical methods. 6th ed. Oxford and IHB. Publishing Co. New Delhi.

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Effect of Season on Histomorphometry of Testis and Epididymis in Nellore sheep

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ABSTRACT

Histomorphometrical changes of testis and epididymis in Nellore sheep were studied in two different groups rainy (August) and winter (December) born aged from'O' day to 8 months. In both the groups, the organisation of germinal epithelium and time of appearance of different cells were studied. Regional differences between the caput, corpus and cauda regions of epididymis were also noticed throughout the growth period.

———x———x———

In breeding rams postnatal traits of testis and expididymis are used to predict its reproductive potential. Varadin *et al.,* (1975) found a marked variation in the histology of testis in lambs born in different seasons. As there is paucity of literature on postnatal development of reproductive organs in native sheep, the present investigation was undertaken.

MATERIALS AND METHODS

A total of 72 Nellore jodepi ram lambs aged from 'o' day to 8 months [36 born in rainy (August) G.I. and another 36 born in Winter (December) G.II] maintained at Livestock. Research Station, Chintaladevi, Nellore district, A.P., were utilized. After castration done at monthly interval tissues were collected from the testis and from caput, corpus and cauda regions of the epididymis and were fixed in Bouins solution for processing by routine paraffin embeding method. Thin sections were cut and stained by H&E method (Humason, 1972). The histological features of germinal cells were studied as per Abdel Raouf (1960). By using occular micrometer the average diameter of the 20 round seminiferous tubules (SFT) was measured. Similarly average diameter, height of epithelium and stereocilia of each region were recorded and compared between two groups. The difference between the means of all the parameters studied was statistically analysed (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

Testis: In both the groups by 5th month, gonocytes revealed degenerative changes with simultaneous appearance of prespermatogonia and spermatogonia and were completely disappeared by 6 months. At 6 months many SFT revealed spermatogonia in group I and few in group II (Baishya *et al.,* 1967). However Ali *et al.,* (1989) reported the same at 4 months of age. Signs of fragmentation of SFT were showed at 7th month leading to formation of lumen (Many SFT in group I and few in group II) The primary spermatocytes appeared in more number of SFT by 7th month in group I than in group II lambs. The spermatids and spermatozoa appeared at 8 months of age in both the groups where as Ali *et al.,* (1989) reported its appearance within 200 days. The delay in these changes indicates in slow onset of puberty and sexual maturity in this breed.

In both the groups mature leyding cells and Sertoli cells were identified at the age of 6th and 7th month respectively (Hassan *et al.,* 1984). The mean diameter of SFT in this study was 34.56±0.45μ in group I and 34.19±0.12μ in group II at birth and at 8 months the same was increased to

189.28±0.82μ and 180.28±0.42μ respectively.

Epididymis: The average diameter of the tubule at birth in caput, corpus and cauda regions respectively was 70.50±0.23μ 72.30±0.13μ and 94.80±0.10μ in group I and 70.85±0.29μ 69.47±0.18μ and 74.62±0.16μ in group II, which increased to 328.12±0.34μ 380.00 ±0.38μ and 389.20±0.31μ in group I and 325.00 ±0.36μ 374.22±0.38μ and 387.75±0.24μ in group II. The difference in the diameter of epididymal tubule between groups, and regions was significant ($P<0.01$) but it was insignificant between the left and right sides of epididymis (Raja and Rao, 1985). At 7th month of age some of the tubules contained spermatozoa in group I while no sperm in group II and by 8th month many tubules contained spermatozoa in group I and few in group II.

At birth, the mean height of the epithelium was greatest at cauda lesser in corpus and least in caput. But at 8th months of age it was greatest at caput lesser in corpus and least in cauda. The different epithelial height after 7 months coincided with the entry of sperms into the epididymal tubules.

In both the groups by one month no stereocilia were discernable in caput and corpus except in cauda. By third month cilia were noticed in caput and corpus regions also. The mean height of the stereocilia in cauda increased from first month to 6 months of age, but decreased by 8 months. In caput and corpus stereocilia continued to increase in height from 3 months of age.

In the present investigation, differences between Group I and Group II lambs might be due to difference in the plane of nutrition, availability of adequate quantity of green grass and environmental temperature. This inadequate nutrition may delay the onset of puberty (Poster *et al.*, 1989). Hence it was concluded that rainy born lambs may be preferred to winter born lambs while selecting the rams for beeding.

REFERENCES

Abdel Raouf S E A,Sharaby M A and Suleiman I O 1989 Studies on the age of puberty of Najdi Rams. Animal Reproduction Science *20:* 67-69.

Ali M M, Rao A R and Singh U B 1989 Postnatal development of testis and epididymis of Deccani rams. Indian Journal of Animal Reproduction *10:* 33-37.

Baishya G, Ahmed S and Bhattacharya M 1987 Development of testis in Assam goat (capra hircus). Indian Veterinary Journal *64:* 24028.

Poster R.A, Ladds P W, Hoffman D and Briggs G D 1989 The relationship of scrotal cercumference of testicular weight in rams. Australian Veterinary Journal *66:* 20-22.

Hassan S G, Yones A A, Ibrahim M K and Nawito M 1984 Some aspects of reproduction in male Bakri sheep. 1. Postnatal testicular development and growth rate. In 10th International Congress on Animal Reproduction and Artificial Insemination, University of Illinois.

Humason G L 1972 Paraffin sectioning and mounting in "Animal tissue techniquies chapter 5. 3rd edition W H Freeman and San Fransisco P[56]

Raja C K S V and Rao A R 1983 Post natal growth of testis and epididymis of brown swiss crossbred bulls. Indian Journal of Animal Science *53:* 73-76.

Snedecor G W and Gochran W G 1967 Statistical methods, 6th edition. Oxford and IHB publishing Co., Calcutta.

Varadin M, Stipanceive L, Pavlovi C D 1975 The significance of seasonal changes in the size of the testis and the diameter of the epididymis in rams. Zucwthy giene *10:* 119-124.

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Fecundity of Preserved Boar Semen With Bts, Kiev and Bl-1 Dilutor

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ABSTRACT

A total of 138 sows were inseminated with semen samples diluted with Belt's Villae Thaw Solution (BTS), Kiev or Belt's Villae Liquid-1 (BL-1) dilutors and preserved for 0, 24, 48 and 72 hours. Mean farrowing percentage with BS, Kiev and BL-1 dilutor was found 61.22, 62.22 and 54;54 respectively. Higher farrowing percentage (69.11) was observed when freshly diluted semen was used which decreases with increase in preservation time. The overall mean litter size with BS, Kiev and BL-1 dilutor was 8.33±0.12, 8.61±0.36 and 8.43±0.38 respectively and there was non significant effect of both dilutors and hours of preservation on litter size at birth. The correlation coefficient between farrowing percentage and mean percentage of seminal characteristics of preserved boar semen were found highly significant.

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Artificial insemination in pigs has the primary objective of genetic improvement with maximum health control at least cost (Walter *et al.,* 1984) but short storage life of boar spermatozoa comes as one of the main hinderence in it's wider application. In India, very limited work on preservability of boar semen (Murthy and Rao, 1975; Rao *et al.,* 1991; Rao *et al.,* 1992) and assessment of fertility following artificial insemination with liquid semen has been done. The present study therefore, was carried out to assess the effect of seminal characteristics of preserved boar semen on farrowing rate and litter size at birth following single insemination.

MATERIALS AND METHODS

Semen samples were collected at 5 days interval with gloved hand from 5 Large White Yorkshire boars in the age group of 1.5 to 2 years following the procedure of Zavos and Liptrap (1987) and were diluted with BS, kiev and BL-1 dilutors. The diluted semen samples were kept separately in 100 ml flexible plastic bottle and preserved at 18°C. The plastic bottles were swirled once daily to prevent agglutination. Sperm motility, live sperm percentage, acrosome morphology, head and tail abnormalities were assessed after bringing the sample to 37°C. Samples showing individual motility of 75 per cent or more were utilized for preservation. Seminal characteristics of preserved semen were assessed at 24 hours interval following the standard procedure. A total of 138 oestrus sows showing the sign of immobilization were inseminated once with 100 ml of diluted semen. Twelve types of semen samples viz. freshly diluted with 3 dilutors and preserved for 24, 48 and 72 hours were used for insemination.

RESULTS AND DISCUSSION

The mean value of seminal characteristics of preserved boar semen showed highly significant correlation with farrowing percentage. The

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average farrowing rate with BTS, Kiev and BL-1 dilutor, irrespective of hours of preservation was recorded as 61.22, 62.22 and 54.54 per cent respectively which is similar to the finding of Murthy (1977). However, higher farrowing rate has been reported by some workers (Blichfeldt, 1989).

Diluted semen with BS, Kiev and BL-1 diluters showed decline farrowing rate (71.43 to53-85 with BS; 69.23 to 55.56 with Kiev and 66.67 to 44.44 with BL-1) along with variation in seminal characteristics with increase in duration of preservation 0 to 72 hrs which may be due to decline in quality of spermatozoa or embryonic mortality (First *et al.,* 1963). Higher farrowing rate (69.11 per cent) irrespective of diluters was observed when freshly diluted semen was used. The lowest farrowing percentage (51.28) was recorded with 72 hours preserved semen and are in accordance to the finding of Vijay Kumar and Iyer (1980) and Johnson *et al.,* (1982).

The overall mean litter size with BS, Kiev and BL-1 dilutors was 8.33±0.12, 8.6±0.36 and 8.43±0.38 respectively which is in close agreement with the findings of Murthy (1977). The difference in litter size might be due to difference in number of insemination, insemination conditions, insemination procedure and genotype of the dam (Peter *et al.,* 1989). Analysis of variance revealed non significant effect of both dilutors and hours of preservation on litter size which is in close agreement with the findings of Vijay Kumar and Iyer (1980) and Blichfeldt *et al.,* (1989).

REFERENCES

Blichfeldt, I., Almlid, T. and Stavne, S.E. 1989, Liquid preservation of boar semen in Kiev or BS. A field comparison. Interntaional seminar on Animal Reproduction, Dublin, 1989.

First, N.L., Stratman, F.W. and Casida, L.E. 1963. Effect of sperm age on embryo survival in swine. Journal Anim. Sc. *22:* 135.

Johnson, L.A. Aalbers, J.G. Willems, C.M.T., Rademaker, J.H.M. and Rexroad, C.E., 1982. Use of boar spermatozoa for artificial insemination. III. Fecundity of boar spermatozoa stored in Beltsville liquid and kiev extenders for three days at 18°C. Journal Anim. Science; *54:* 132-136.

Murthy, P.R. and Rao, A.R. 1975. Preservation of boar semen. Indian Vet.J., *52(6):* 415-420.

Peter, W., Mudre, K., Conrad, F. and Zeuner, A. 1989 Measures to improve liquid preservation of boar semen. International seminar on Anim. Reprod. 1969, Dublin.

Rao, B.V., Venkatamuni Chetty, A., Ramachandraiah, S.V. and Sreeraman, P.K. 1991. Preservation of native boar semen. Indian J. Anim. Reprod., *12(2):* 148-150.

Rao, M.M. Ramachandraiah, S.V., Vekatamuni Chetty. A. and Sreeraman, P.K. 1992. Studies on crossbred boar semen characteristics and preservation. Indian J. Anim. Reproduction, *13(2):* 141-142.

Vijay Kumar, V. and Iyer, C.P.N. 1980. Extenders for preservation of boar semen. Kerala Journal Vety. Science. *11:* 215.

Walters, J.R., Hooper, P.M. and Green, C.G. 1984. The utilization of artificial insemination in comercial pig improvement E.A.A.P., World Review of Animal Production. Vol.XXII No.2. 1986.

Zavos, P.M. and Liptrap, D.O. 1987. Procedures for collection, evaluation, dilution and artificial insemination of boar spermatozoa, Agril. Practice Swine Reproduction, *8(3):* 268.

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Comparative Study of Polyovular Follicles in Goat and Sheep Ovary

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The number of ovulations during oestrous cycle in farm animals is determined by (1) number of maturing follicles, (2) hormonal profile, (3) rate of growth and selection of follicles, and (4) follicular atersia (Draincourt, 1991: and; Guraya, 1996). Another important aspect of bizzare occurrence is the polyovular follicles which have not received much attention. These follicles are characterized by containing more than one oocyte per follicle and may affect the number of ovulations per cycle. Polyovular follicles in the ovaries of goat and sheep are described in this paper.

In the goat ovary, some binucleate oocytes were observed in the primordian follicles. These healthy Follicles were characterized by possessing two nuclei with two distinct nucleoli within one oocyte (Figs. 1&2). Biovular and triovular follicles were also observed at the same stage. These polyovular follicles were enclosed within one Follicle wall (Figs. 3&4). In the sheep ovary, triovular follicles were also observed at primordial stage (Fig.5) in addition to biovular follicles. A few biovular follicles were also seen at two to three layered follicular development stage in both ruminants (Fig. 6). Such types of abnormal Follicles have also been reported in the human (Gougeon, 1981), mole rat (Kaur, 1983), the house rat (Guraya *et al.*, 1987) and rabbit (Al-Mufti *et al.*, 1988).

Binucleate ova may be formed during early development of the ovary when primordial germ cells proliferate by mitotic activity to form numerous female germ cells (Guraya, 1996, 1997). During this process in some primordial germ cells, the nucleus may undergo mitotic division to form two nucleoli, which is not accopanied by cytokinesis, resulting in the formation of binucleate female germ cell or oocyte which gets associated with somatic (or follicle cells) to form primordial oocyte by completing the early stages of meiosis in both the nuclei. It is also possible that binucleate oocyte may be formed by the fusion of two adjacently placed primordial germ cells in the early stages of ovarian development. Multiovular follicles in the ovaries of sheep and goat appear to be formed by the enclosure of more than one primordial germ cells or primordial oocytes by the follicular wall. Morphogenetic factors involved in the formation of polyovular follicles remain to be determined at the cellular and molecular level. It also remains to be determined whether polyovular follicles can ovulate more than one egg in response to various hormonal treatments which are being used for superovulation (Guraya, 1996). Generally polyovular follicles undergo atersia.

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REFERENCES

Al-Mufti, W., Bomsel-Helmreich, O. and Christides, J. P. 1988. Oocyte size and intrafollicular position in polyovular follicles in rabbits. Journal of Reproduction and Fertility, B2 *(1):* 15-26.

Draincourt, M.A. 1991. Follicular dynamics in sheep and cattle. Theriogenology, *35(1):* 55-79.

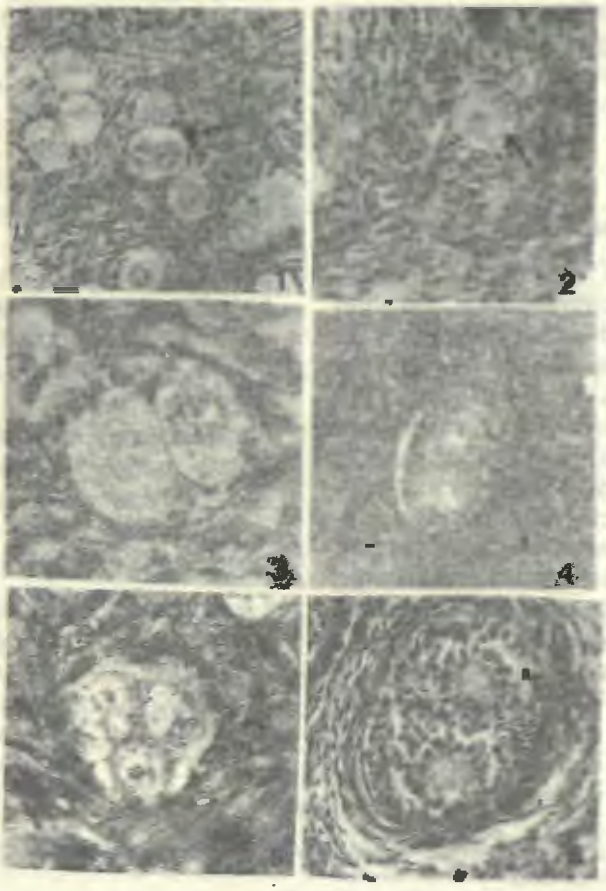
Gougeon, A. 1981. Frequent occurrence of multiovular folicles and multinuclear oocytes in human ovary, Fertility Sterility. *35(4):* 417-422.

Guraya, M., Kaur, P. and Guraya, S.S. 1987. Morphology of the house rat (*Rattus rattus*) ovary during the esterous cycle. Z. Mikrosk Anatony Forsch (Leipz). *101(5):* 815.828.

Guraya, S. S. (1996). Cellular and molecular Biology of Gonadal Development and Maturation in Mammals: Biomedical Implication. Narosa Publishing House, New Delhi and Springer - Verlag. Heidelberg.

Guraya, S. S. 1997. Ovary development, structure and functioninmammals with focus on buffaloes and cattle. ICMR Publications, New Delhi.

Kaur, P. and Guraya, S. S. 1983. Follicular growth and kinetics during the estrous cycle, pregnancy and postpartum in the Indian mole rat (*Bandicota bengalensis*). American Journal of Anatomy, *166(4):* 469.482.



1. A binucleate ovum (arrow) lying in a number of primordial follicles in goat ovary. x 400. 2. A binucleate ovum (arrow) showing distinct cytoplasmic demarcations. X 400. 3. Biovular single layered follicle in goat ovary; oocytes are indifferent stages of development. x 1000. 4. Biovular goat single layered follicle in goat ovary. x 400. 5. Magnified version of triovular primordial follicle in sheep ovary. x 1000. 6. Biovular atretic follicle; both oocytes degenerating in sheep ovary. x 400

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Uterine rupture following incomplete cervical dilation in a gaddi goat - a case report

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Dystocia due to failure of cervical dilation is seen occasionally in the cows and ewes and very rarely in other domestic animals (Roberts, 1971). There is only one available report regarding ring womb and uterine rupture in a goat (Sundaravadanan *et al.,* 1995). The present communication places on record the rupture of gravid uterus following incomplete cervical dilation in a gaddi goat.

CLINICAL FINDINGS-TREATMENT

A four year old, primipara, pregnant gaddi goat having completed the gestation period was presented to the polyclinic with a history of dystocia. As per owner, the animal was restless and exhibited mild, intermittent and irregular labour pains since last 24 hours, without further progress to second stage of labour. Except for a thick string of cervical mucus no other discharge was observed. Transabdominal bellotment revealed a fetus in the abdomen. On per-vaginal examination the cervix was one finger open. On repeated examination the cervix failed to dilate further and the case was suspected for incomplete dilation of cervix.

Keeping in view the clinical findings, laprotomy was performed on left flank under local infilteration and field block anaesthesia using lignocaine (BAIF). On exploring the uterus, it was found to be empty, involuting and there was a complete tear along the entire length of lesser curvature of the right horn. Through the tear, water bags enclosing fetus were extended into the abdominal cavity. A very few placentomes formed attachment between uterus and allantochorion. There was no putrefaction of the contents of the uterus. The water bags were ruptured and a normal sized, dead male kid was delivered. After removing the placenta, uterus and surgical incision site were sutured in a routine manner. Intravenous infusion of 500 ml of daxtross normal saline (wochkard) and 8 mg of dexamethasone (Indian Immunologicals) were given during surgery. Adequate postoperative care was taken till recovery.

Incomplete dilation of cervix is one of the cause of uterine rupture in bovines (Khar *et al.,* 1993) which might be because of convulsive limb movements and respiratory failure before death (Arthur, *et al.,* 1989).

REFERENCES

Arthur, G.H., Noakes, D.E. and Pearson, H. (1989) Veterinary Reproduction and Obstetrics. 6thEdn. ELBS Bailliere Tindal, Great Britain. pp 323.

Khar, S.K., Mannari, M.N. and Jit Singh. (1993). Ruminant Surgery, Ed., R.P.S. Tyagi and Jit Singh, CBS Publishers and Distributors, Delhi.

Roberts, S.J. (1971). Veterinary Obstetrics and Genital Diseases. 2nd Edn. CBS Publishers and Distributors, Delhi. *pp* 292-93.

Sundarvandanan, V.K., Shivaprakash and Honnappagol, S.S. (1996). Ring womb and uterine rupture in a goat. *Indian. Vet. J. 73:* 324-25.

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Rupture of uterus following uterine torsion in a buffalo - a case report.

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Spontaneous rupture of the bovine uterus followed by partial or total displacement of foetus in the peritoneal cavity is an uncommon complication of late pregnancy (Parkinson, 1974; Arthur *et al.,* 1982, Haque, 1991). The present communication records a case of uterine rupture with foetus in the peritoneal cavity in a buffalo.

History and clinical examination: A 5 years old buffalo was brought to the clinic with an history of dystokia for last 10-12 hr. The animal was depressed, restless and reluctant to move. On examination animal was found to be having 180° post-cervical uterine torison. Therefore, it was decided to perform cesarean section to deliver the foetus.

Surgical management: The buffalo was tranquillized with detomidine hydrochloride 1.5 ml (20 µ g/kg body wt. i/v). Surgery was performed on left side just parallel to milk vein after infiltration of site with 2% xylocaine hydrochloride. Following leprotomy the gravid right uterine horn was found to have involuted to a considerable degree and the dead foetus was found partially slipped into the peritoneal cavity through a tear on the ventro-latereal aspect. Foetus was removed. Blood clots and fibrin were cleared from the abdominal cavity by rinsing and syphoning with normal saline. Tear on uterus was sutured before routine suturing of cesarean section. Supportive therapy was given to the animal. Skin sutures were removed on 10th day of surgery. The animal recovered uneventfully and was discharged as treated.

Discussion: Uterine rupture is usually the result rather than the cause of dystokia (Parkinson, 1974 and Haque, 1991), the predisposing cause being uterine torsion, breech presentation and abnormal foetal postures. In the present case, the predisposing cause was the post-cervical uterine torsion.

Uterine rupture during parturition may result in considerable uterine haemorrhage and thus hypovolumic shock (Arthur *et al.,* 1982). However, Parkinson, (1974) opined haemorrhage from the uterine wound at the time of rupture, though inevitable, but not fatal as rapid involution of uterus after relase of foetus in the late pregnancy may effictively control the haemorrhage. In the present case evidence of moderate bleeding was present, but was not fatal.

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REFERENCES

Arthur, G.H., Noakes, D.E. and Pearson, H. (1982). Veterinary Reproduction and Obstetrics, Baillere Tindall, London p 261-275.

Haque, M(1991). Repture of uterus with displacement of foetus into the pertioneal cavity in a cow-a case report. Indian Vet J. *68:* 271-272.

Parkinson, J.D. (1974). Bovine cesarean section in general practice. Vet. Rec. *95:* 508.

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Laparoscopic Diagnosis of Perimetrial Adhesion in a Cow

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Perimetritis which is a sequelae of peri and / or post parturient complication is not uncommon in cows and is characterized by varying amount of adhesions (Roberts, 1971). A parous crossbred cow with a history of repeatedly bred but not conceiving was brought to the infertility clinic of Madras Veterinary College Hospital. Anamnesis revealed that the animal suffered with retention of placenta followed by metritis.

CLINICAL EXAMINATION

Clinical examination showed the uterus in the pelvic cavity but the contour of the uterine horn was not completely traceable. The mobility of the uterus was also restricted. The animal was subjected to laparoscopic examination for further investigation and confirmatory diagnosis.

LAPAROSCOPIC EXAMINATION

The animal was prepared by withholding feed and water for 48 hours. On the day of examination the right flank was prepared in a routine manner for laparoscopic examination. The animal was sedated with 20mg of xylazine hydrochloride (Indian Immundogicals) administered intramuscularly. At the middle of the flank about 10ml of lignocaine hydrochloride (Astra) was infiltrated to bring about local analgesic effect. A skin incision about 1 cm length was made and a 11mm trocar and cannula was inserted by thrusting the tip about 45° angle into peritoneal cavity caudally. Following withdrawal of the trocar a 30° oblique angle 10mm Hopkins Telescope (Karl storz, GmbH, Germany) connected with light source and also with an endocamera and T.V. monitor was introduced for a detailed examination of the uterus. After laparoscopic examination skin wound was closed with a single mattress suture using black braided silk.

RESULT AND DISCUSSION

On laparascopic examination perimetrial adhesion was visualized around the walls of the uterus. There was marked adhesion between the uterine body and rectum. Fibrin strands extending from the rectum to the uterus were clearly observed (Fig. 1). A fibrinous membrane covering the uterus was also visualised (Fig.2) and this could be the possible reason for the inability to feel the contour of the uterus.

Laparoscopy was an effective procedure for examination of the pelvic organs and for diagnosis of reproductive tract abnormalities in cows (Maxswell and Kraemer, 1980). In the present case also laparoscopic examination helped in the visualization of the genital organs and to confirm the extent and nature of perimetrial adhesions.

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Fig 1

Fig 2

Fibrin strands and fibrinous membrane extending from rectum to uterus

REFERENCES

Maxwell D.P. and D.C. Kramer, 1980. Laparscopy in cattle in "Animal laparoscopy". Eds. Harrison. R.M. and D.E.Wildt. William and Wilkins, pp. 155.

Roberts, S.J., 1971. Cited in Veterinary Obstetrics and Genital Diseases (Theriogenology). Edt. Roberts S.J., Edn. II. CBS Publishers & Distributors (India), pp. 485.

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A note on mummification of feotus in cattle

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Mummification of feotus is one of the important gestational disorder in livestock where feotus dies in the dam's uterus and remain insitu beyond the normal length of gestation (Roberts, 1962). Factors which initiate events leading to mummification of feotus in cattle are uncertain. Torsion of uterus (Nillson, 1969) and genetic relationship (Davidson and Roberts, 1961). however, have been found to be associated with the death and mummification of feotus. In the present paper two cases of mummification of feotus, one in Hariana and other in crossbred cattle have been reported.

Two casses of mummification of feotus one each in Hariana and crossbred cow (1/2F.1/4B.1/4H) was recorded at the Institute's dairy herd. The animals were checked and confirmed pregnant by rectal palpation 60 and 120 days following insemination but could not deliver calf after completing the normal gestation period. Rectal palpation of these animals revealed thickened uterine wall having no pregnancy tone, or feotal membranes, cotyledons and presence of feotal fluid. The uterus was contracted and encapsulated on the feotus. The feotus was palpated as a hard, dry leather-like lump inside the uterus.

To expell out the mummified feotus animals were treated with single intramuscular injection of PGF_2 α (25 mg Lutylase, Upjohn Limited, West Sussex). Animals were also given 50 mg stilbestrol and 50 IU Oxytocin intramuscularly once daily for two days. On 3rd day afternoon chocklate coloured thick mucous discharge was noticed coming from vulva. On rectal palpation a hard lump was found in the birth cannal. The mass was removed manually by slight traction.

Out of two animals Hariana cow was culled from the herd. The crossbred cow came into estrus 36 days following expulsion of mummified feotus. She was not served and given breeding rest. She again came to estrus after 21 days and was inseminated. The animal did not show estrus following insemination. Animal was found pregnant on examination 60 days after insemination. After normal gestation delivered a normal calf. Prostaglandin F_2 α has been used for the treatment of mummification in cattle by Tamuli *et al.* (1987) and Ramachandraiah and Reddy (1992).

It may be concluded that PGF_2 α alongwith stilbestrol and Oxytocin can successfully be used for expulsion of mummified feotus in cattle. The fertility of dam following expulsion of mummified feotus was normal.

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REFERENCES

Davidson, J.G. and Roberts, S.J. (1961). Fetal mummification : A study among dairy cattle. Cornell Vet. *51:* 34-36.

Nillson, L.S. (1969). Fetal mummification - a new twist. J. Am. Vet. Med. Assoc. *155:* 1560-1561.

Ramachandraiah, K. and Reddy Mohan, A.R. (1992). Dinofertin therapy for mummified feotus. Indian Vet. J. *69:* 452-453.

Roberts, S.J. (1962). The enigma of fetal mummification. J. Am. Vet. Med. Assoc. *f140:* 691-698.

Tamuli, M.K., Rajkonwar, C.K. and Borgohain, B.M. (1987). Mummification of feotus in a crossbred Jersey cow. Indian Vet. J. *64:* 608-609.

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Schistosomus Reflexus with Perosomus Elumbis in Holstein Friesian Cow

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Dystokia occurs most commonly with monsters (Parkinson 1974). Present article records a case dystokia caused by schistosomus reflexes with perosomus elumbis condition.

CASE HISTORY AND OBSERVATIONS

A five years old Holstein-Friesian cow with symptoms of dystokia was observed at Livestock Production Research Farm of this Institute. The cow was in third parity and calving was normal in earlier two pregnancies. Vaginal examination revealed completely relaxed cervix with exposed visera protruding from vagina. Foetus was in posterior presentation and hip lock position. Attempt was unsuccessful to correct and relieve dystokia by mutation and forced traction. Later it was decided to perform caesarean section. The operation was performed under sedation and local analgesia following standard surgical proceedure. Postoperative antibiotic cover and dressing of the incision was carried out and the cow showed uneventful recovery by 10th day.

DESCRIPTION OF THE MONSTER

It was still born male calf about 32 kg in weight. The development of thoracic and abdominal cavity was incomplete. The skin of both the sides was united at umblicus. Vertebral column was not developed beyond thoracic region. All the internal organs including heart and lungs were protruding out from dorsal aspect of abdominal cavity. The organs were apparently normal. The limbs were ankylosed and both the hind limbs were flexed from fetlock joint. Abdominal and pelvic cavity were open on dorsal aspect.

Present anomaly was identified as Schistosomus reflexus with Perosomus elumbis condition according to the classification of Roberts (1971). Schistosomus reflexus with other anomalies has been reported in cow (Balasubramanian *et al.,* 1991 and Padma Rao, *et al.,* 1993) and in buffalo (Shastry and Murthy, 1984).

REFERENCES

Balasubramanian, S., Thilagar, S, Kathiresan D. and Pattabiraman S.R. (1991) Indian Vet. J. *68:* 567.

Padma Rao, V. Ramachandraiah, K., Mohan Reddy, A.R., Venkata Subbiah, D. and Chandrasekhar, B. (1993). Indian Vet J. *70:* 1083.

Parkinson, J.D. (1974) : Vet Rec. *96:* 508.

Roberts, S.J. (1971) : Veterinary obstetrics and genital diseases 2nd Edn. Ithaka New York.

Shastry, G.J.S. and Murthy, J.S. (1984). Indian Vet. J. *61:* 1084.

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Prolapse of Uterus in a Mare

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Prolapse of uterus is a common postpartum complication in bovines and ovines, occasional in swine and is considered to be rather infrequent in equines, canines and felines (Roberts, 1971). The occurrence of this problem in equines may be due to forced extraction of the dry fetus which predisposes to violent or strong tenesmus, retained placenta especially at the ovarian pole of the non gravid horn or mild to moderate straining after abortion (Roberts, 1971, Arthur, 1986) or occur during immediate post partum period (Howlett, 1981, Hastinap and Miller, 1983 and Krishnaswamy *et al* 1985)

The present report describes a case of complete uterine prolapse following abortion due to colic pain in non-descript draft mare.

CASE REPORT:

A seven year old non-descript draft mare was presented with complete uterine prolapse (Fig.) and having abdominal pain. The history revealed that the animal exhibited abdominal pain two-days before and aborted a four and a half month old foal on same day evening. The fetal membranes were expelled immediately following abortion with complete eversion of the uterus simultaneously. The prolapsed part of the uterus was replaced two times by quack but it relapsed after 4-5 hours. The case was presented at the Animal Reproduction Clinic, College Of Veterinary Sciences, Lahore, two days after this happening. The prolapsed portion was grossly contaminated, dried and lacerated. The animal was dull, depressed, anorectic and febrile.

TREATMENT:

The animal was restrained with the help of side line and sedated with 150 c.c. chloralhydrate (10% solution) intravenous. The tail was covered with long plastic sleeve and epidural anesthesia was induced with 6 ml. of 2% xylocaine hydrochloride. The perineal region was cleaned thoroughly with the help of soap and water, whereas, the prolapsed uterus was thoroughly washed 1% potassium permagnate solution. Then antiseptic cream (Burnol) was liberally applied on it. The prolapsed portion was subsequently replaced by lifting it up at the vulvar level and pushing inside with palm pressure as in the bovines and ovines. The vulvar lips were sutured by horizontal mattress pattern with silk thread, closing the two-third of the vulvar opening. After replacement the mare was drenched one liter simple oil mixed with 15 gm. chloralhydrate and 30 c.c. turpentine oil. Tetanus antitoxin (10,000 i.u.) was injected intramuscular.

Twenty four hours later, the mare was reported to be eating and drinking normally. She was also reported to pass the faeces and urine normally. There was no evidence of abdominal pain or straining. The mare was kept under observation for five days. During this period the Combiotic injection 5 gm. (Benzyl Penicillin 500,000 i.u., Procaine Penicillin 150,0000 i.u., Streptomycin Sulphate 5 gm.) diluted with 100 c.c. distilled water was administered daily in the uterus, whereas, 30 ml.

Genta-50 (Gentamycin Sulphate 50 mg/ml) was administered intramuscular for first three days. The sutures were removed after one day.

The animal was discharged on 6th day after uneventful recovery.

Fig. Mare with complete uterine prolapse

REFERENCES

Arthur, G.H. 1986. Veterinary reproduction and obstetrics. 6th Ed. Bailliere Tindall, London, pp 298-299.

Hastings, J. and Miller, R. 1983. Uterine prolapse in a mare. VM/SAC., *78:* 1118-1119.

Howlett, J.R. 1981. Complete uterine prolapse in a mare. VM/SAC., *77:* 655-656.

Krishnaswamy, A., Khar, S.K.and Sharma, A.K.1985. Uterine prolapse in a mare. Indian J. Anim. Reprod., *6(1):* 93-94.

Roberts, S.J. 1971. Veterinary obstetrics and genital diseases. 2nd ED. Ithaca, New York, pp 308.

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Changes in Serum Cholesterol Levels During Pregnancy and Post Partum in Sheep

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Cholesterol is an important metabolic compound present in membranes, lipoprotein and body fluids. It is synthesized in the liver and act as a precursor for steroid hormones and bile acids. During pregnancy and at the start of lactation, changes in blood constituents occur (Hafez 1993). Okab *et al* (1993) have observed seasonal variations of cholesterol during pregnancy and parturition. In the present investigation, the serum cholesterol levels were estimated during pregnancy at parturition and during post partum periods.

MATERIALS AND METHODS

A study was conducted on 12 Madras Red Sheep maintained at Liverstock Research Station, Kattupakkam. The animals were allowed for grazing outside during most part of the day and housed in semi open barn during night. They were fed with 300 g of concentrate mixture and offered water *ad libitum*. Blood samples were collected by a jugular vein puncture and the clear serum samples were used for analysis. Serum cholesterol was estimated colorimetrically by the method of Wybenga *et al* (1970). Blood samples were collected on the day of mating and on 30, 60, 90, 120, 140 days of pregnancy and on the day of paturition. The post partum levels of cholesterol was estimated on 1, 5, 10, 15, 20 and 30 days after parturition. Statistical analysis were carried out by the method of Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The results obtained in the present investigation are presented in Table 1. The mean cholesterol in the ewes on the day of mating showed a value of 112.33±0.24 mg/dl. Nazki and Rattan (1991) have observed a value of 99.99 to 103.92 (mg/dl) during different seasons. The serum cholesterol level in the present study showed a raise upto 3 months of gestation and a fall there after. Krajni Nakova *et al* (1993) have observed similar findings in sheep during pregnancy. Okab *et al* (1993) observed highest values in sheep on 135 days of pregnancy with a fall in cholesterol level with advancing pregnancy. Sahukar*et al* (1985) reported similar findings in crossbred cows and attributed this to hormonal influences. Lowest value of serum cholesterol (90.30±0.65 mg/dl) on the day of parturition which was highly significant agreed with the earlier findings of Okab *et al* (1993).

On the first day after parturition the level was 92.27±0.20 mg/dl. Subsequently the cholesterol level observed on 5th to 30th day after parturition varied from 102.93±0.37 to 109.24±0.16 mg/dl. There was no significant difference observed between days after parturition where as the values were higher during pregnancy with a peak value of 119.04±0.28 mg/dl on the 90th day. Blum and Kunz (1981), Gerloff*et al* (1986) and Guyton (1996) concluded that the low level of thyroxine may be the attributory factor for reducing the serum cholesterol level during pregnancy in cows.

Table 1. Serum Cholesterol during Pregnancy Parturition and post partum period in sheep

Status	Range mg/dl	Mean mg/dl	SE
Day of mating	103-114	112.33	0.24
30 days of pregnancy	106-112	111.23	0.14
60 days of pregnancy	106-126	118.12	0.15
90 days of pregnancy	94-133	119.04	0.28
120 days of pregnancy	100-122	116.20	0.16
140 days of pregnancy	96-118	109.84	0.26
Day of parturition	87-98	90.30**	0.65
One day post partum	77-110	92.27**	0.20
5 day post partum	84-126	102.93	0.37
10 day post partum	83-114	103.80	0.20
15 day post partum	105-114	110.03	0.16
20 day post partum	105-112	108.70	0.15
30 day post partum	106-114	109.24	0.16

** $P<0.01$

REFERENCES

Blum J.W. and Kunz.P, 1981. Effect of fasting on thyroid hormone levels and kinetics of reverse triiodothyronine in cattle. Acta.Endocrinologica *98:* 234-239.

Gerloff, E.J, Hardi, T.H, Wells W.W, Nachreiner, R.F. and Emery. R.S. 1986. Inositol and hepatic lipidosis. Effect of inositol supplementation and time from parturition on serum insulin, thyroxine and triiodothyronine and their relationship to serum and liver lipids in dairy cows. J.Anim. Sci., *62:* 1693-1702.

Guyton, A.C, and Hall J.E.,1996. Text book of Medical Physiology. 9th Edi USA, Prism Books Pvt. Ltd., Banglore, India.

Hafez, E.S.E, 1993. Reproduction in farm animals. 6th Edi. Lea and Febiger Publisher, Malvern PA, USA.

Krajni Nakova, M., Bekeova, E., Hendrichovsky. V. and Maracek. I, 1993. Concentrations of total lipids, cholesterol and progesterone during oestrus synchronization and pregnancy in sheep. Vet. Medicina.*38:* 349-357.

Nazki, A.R. and Rattan, P.J.S., 1991. Some hormone and biochemical characters of blood in sheep as related to different seasonal environments. Indian Vet.J.,*68:* 28-32.

Okab, A.B, I.M. Elebanna, M.Y. Mekkawy, G.A. Hassan, F.D. El-Nouty and M.H. Salem, 1993. Seasonal changes in plasma thyroid hormones, total lipids, cholesterol and serum transaminases during pregnancy and at parturition in Barki and Rahmani ewes. Indian J. Anim. Sci.,*63:* 946-951.

Sahukar, C.S, Pandit, R.K, Chauhan, R.A.S. and Porwar, M.L. 1985. Cholesterol and Alkaline phosphates during various reproductive phases in crossbred cows. Indian J. Anim. Sci.,*55:* 421-423.

Snedecor, G.W. and W.G. Cochran, 1967. Statistical methods. 6th Edi., Oxford, IBH Publishing Co., Calcutta, India.

Wybenga, D.R. and R. Pileggi, 1970. Clin. Chem., 16,980.

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Morphological Evaluation of Bull Semen

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Morphological Semen Evaluation:

Traditionally morphological sperm evaluation is performed on stained smears. However, artefacts may arise during the dry preparation like ruptured tails and heads, acrosome damages and broken necks. Many staining methods have been described and used in laboratories all over the world. It must be noticed that different staining methods give results which are not directly comparable. At the Dept of Obstetrics and Gynaecology in Uppsala the carbol-fuchsin method according to Williams (1920) is used, which was introduced by Lagerlof (1934). This stain gives very clear contours of the shape and size of the sperm while details like the acrosome, mitochondrial sheath and cytoplasmic droplets are not so distinct.

Simple and rapid methods for field use are based on so-called negative staining where a mixture of semen and inactive material (Indian ink, opal blue, nigrosine) is smeared on a glass. The unstained sperm stand out as bright particles against a dark background. The Indian ink method was first used. (Blom, 1950a). Instead of ink it may be preferable to use nigrosin which gives nicer preparations. It is also possible to mix eosin and nigrosin in the same stain and use the stained smears both for evaluation of morphology and the frequency of dead sperm (Blom, 1950b, Hancock, 1952, 1957). These rapid methods give a relatively good estimation of the number of abnormal sperm and proximal and distal cytoplasmic droplets.

For phase contrast microscopy wet preparations are prepared by diluting semen with buffered formalin (Hancock, 1957). The diluted semen can be kept for a long time in refrigerator without morphological changes and can also be used for preparation of dry smears (Sekoni et al, 1981). Many of the finer details of the sperm will be seen in phase contrast microscopy of wet preparations. The acrosome with the apical ridge and the equatorial segment are clearly visible. In a similar way abnormalities of the midpiece will be seen clearly. In case of rupture of the tail the individual fibrils can be seen. The cytoplasmic droplet is easily seen. It is on the other hand difficult to evaluate the shape and size of the sperm head as the sperm are turning around in the wet preparation and can show sometimes the edges and sometimes the flat side. Examination of both stained smears in light microscope and wet preparations in phase contrast microscope are therefore two methods that will complement each other in an excellent way.

Classification of pathological sperm

The classification of the sperm is done not only to differentiate between normal and abnormal sperm but also to describe the different types of abnormalities. The reaction of the germinal epithelium to different damaging factors is largely unspecific which means that the same type of pathological sperm are caused by disturbances of different nature. Under practical conditions this type of abnormalities may be described as *unspecific pathological sperm*. This group is made up of changes of the shape and size of the head, abnormalities of the mid-piece and tail and position of the

cytoplasmic droplet. It is these types of unspecific changes which we usually refer to when we talk about pathological sperm. On the other hand there are cases when only one special structure is defective in a large number of sperm, e g the acrosome, the nuclear chromatin or the midpeice, while the rest of the sperm morphology is normal. These types of abnormalities should preferably be called *specific sperm defects*. These are as a rule congenital disturbances of a certain phase of the spermateliosis, which gives a typical morphological defect.

The first more comprehensive description of pathological sperm in the bull was published by Lagerlof (1934). He classified the sperm morphologically into different groups (fig). Lagerlof pointed out that even so-called normal sperm showed a certain degree of morphological variation. It is therefore necessary before a semen smear is examined in detail to survey the slide to establish if the bull has a special type of sperm head morphology. Only obvious deviations from a bull's typical sperm head type should be registered as pathological.

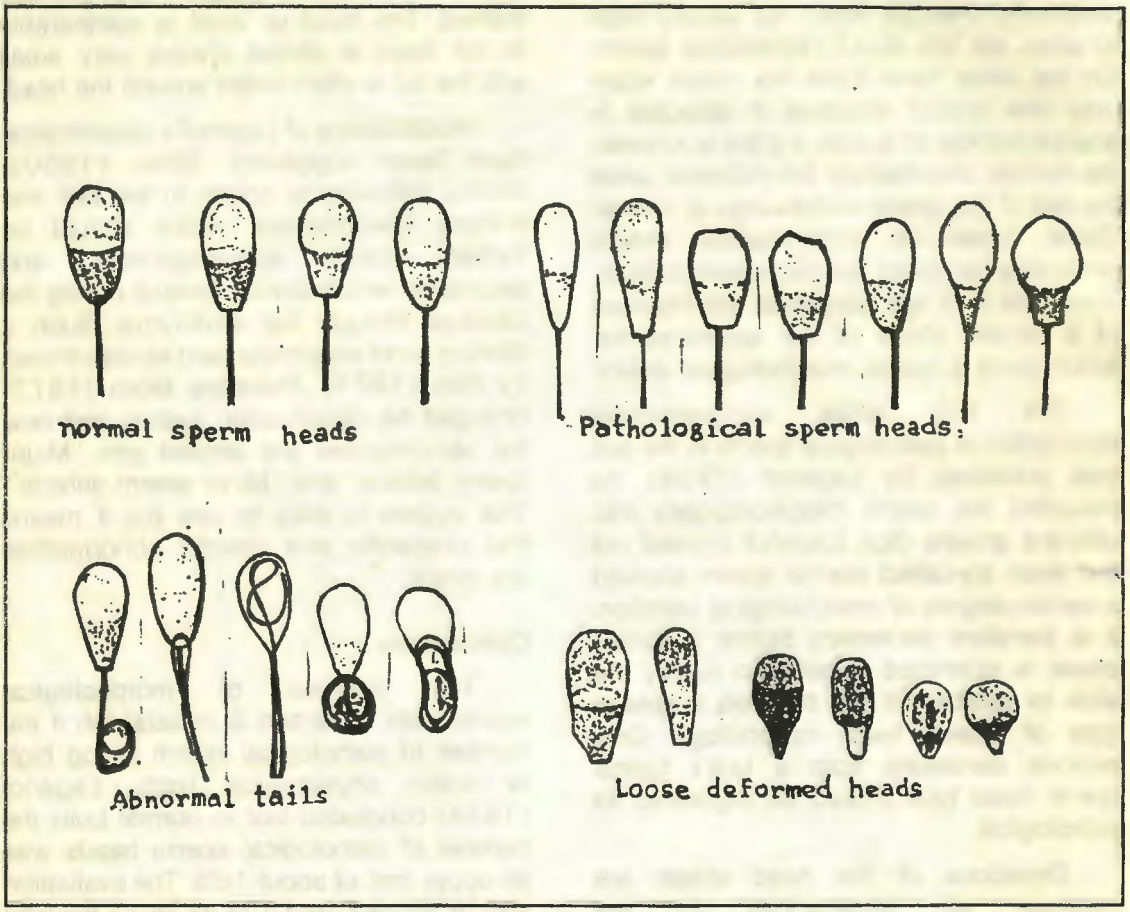
Deviations of the head shape are common at disturbances of the spermiogenesis and are important for the diagnosis. Among the deviations are narrow heads, narrow at the base, pear shaped and heads with abnormal contour. Abnormalities of the midpiece and tail are not as common in the bull as in other species. Loose deformed heads have probably lost the tail during the passage through the tubular system.

Undeveloped sperm are different types of abnormal sperm which are formed during disturbances of the spermateliosis. The heads are often very defective and strongly stained. The head or what is comparable to the head is almost always very small and the tail is often coiled around the head.

Modifications of Lagerlof's classification have been suggested. Blom (1950a) divided pathological sperm in the bull into primary abnormalities, which should be formed during spermiogenesis, and secondary which should develop during the passage through the epididymis. Such a division is not principally valid as was shown by Rao (1971). Therefore Blom (1973) changed his classification system and now the abnormalities are divided into "Major sperm defects" and "Minor sperm defects". This system is easy to use but it means that unspecific and specific abnormalities are mixed.

Conclusions

The purpose of morphological examination of semen is to establish if the number of pathological sperm is too high or within physiological limits. Lagerlof (1934) concluded that in normal bulls the number of pathological sperm heads was an upper limit of about 18%. The evaluation should not be based exclusively on the total number of abnormal sperm heads but should also take into consideration what types of abnormalities are most frequent. Pear-shaped, narrow at the base, undeveloped and loose deformed heads are more serious abnormalities than the others. If they dominate the spermiogram the conclusions must be more cautions even if the total number of abnormalities is close to the upper physiological limit. The final evaluation must also take into consideration other semen characteristics like motility and total sperm number of the ejaculate.



REFERENCES

Blom,E. 1950 a. On the evaluation of bull semen with special reference to its employment for artificial insemination. Thesis, Copenhagen.

Blom,E. 1950 b. En hurtig-farvingsmetode till adskillelse af levende og dode spermier ved hjaelp of Eosin-Nigrosin. Nord.Vet.-Med.*2*, 58-61

Hancock, J.L. 1952. The morphology of bull spermatozoa. J. Exp. Biol. *29*, 445-453.

Hancock, J.L. 1957 The morphology of boar spermatozoa. J.R. Micr. Soc. *76*, 84

Lagerlof, N. 1934. Morphologische Untersuchungen uber Veranderungen im Spermabild and in den Hoden bei Bullen mit verminderter oder aufgehobener Fertilitat. Acat path. microbiol Scand. Suppl. 19 Thesis. Stockholm.

Rao, A.R. 1971. Changes in the morphology of sperm during their passage through the genetal tract in bulls with normal and impaired spermatogenises. Thesis, Stockholm.

Sekoni, V.O., Gustafsson, B.K. & Mather, E.C. 1981. Nord. Vet. -med.*33*, 161-166.

Williams, W.W. 1920. Cornell Vet. *10*, 87-94.

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Testosterone Levels in Buffalo Bulls

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The purpose of the present investigation was to know the basal, mean and peak levels of Testosterone (T) in the Surti buffalo bulls and study the pattern of 'T' secretion.

MATERIALS AND METHODS

Studies on the tstosterone levels in six Surti buffalo bulls aged between 61/2 to 7 years were made during the six hour period of study and based on 36 sera samples. Blood samples were drawn at hourly interval from each bull commencing from 8 a.m. Radio-Immuno-Assay procedure was employed for the estimation of levels of 'T'. At the Reproductive Biology Research Unit, GAU, Anand. The study was undertaken during winter months of peak breeding season (November and December, 1990).

RESULTS AND DISCUSSION

Based on 36 sera samples in 6 buffalo bulls, the basal, mean and peak levels of testosterone were 1.97±0.43, 3.59±0.28, and 5.12±0.39 ng/ml respectively. Testosterone secretion in intact males is not tonic but is characterised by episodic pulses. This pattern of secretion varied significantly, ($P<0.05$) between bulls and between hours. This may probably due to the age, reproductive status, health, external environment and other factors. Mean testosterone levels between the buffalo bulls varied significantly ($P<0.05$) from 2.443±0.327 to 4.850±0.352 ng/ml.

Number of peaks in 'T' levels observed to vary from one to three. The mean time interval between two peaks was 150 minutes.

Kamonpatana (1984) found the number of 'T' peaks varying between 1 to 10 during 24 hours where the blood sampling was done every 15 minutes.

The mean basal 'T' level of 1.97±0.43 ng/ml under the present study compares well with the levels already reported (Parera *et al.*, 1979, Chandraprateep *et al.*, 1981 and Gupta *et al.*, 1984).

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REFERENCES

Chandraprateep, P., Kamonpatana, M., Lahachit, H., Kunawongkrit, A., Verokul, P., Bodhipaksha, P., and Nagrem Suriyaraj, C. (1981), Circadian variations of plasma LH and testosterone in adult Swamp buffalo bulls, *Theriogenology, 15:* 161-171.

Gupta, R.C., Sharma, A.K., and Khurana, N.K. (1984), Testosterone levels and libido in buffalo bulls. Proc. 10th International Congr. AR & AI., Vol. II, pp. 271-272, 1984 USA.

Kamonpatana, M (1984), Application of Hormone assays and endocrine pattern in buffalo, Proc. 10th International Congr. AR & AI., Vol. IV, pp. -1-10, 1984 USA.

Perara, B.M.A.O., Pathiraja, N., Matha, M.X.J., and Weerasekera, D.A., (1979), Seasonal differences in plasma testosterone profiles in buffalo bulls, *Theriogenology 12*:33-38.

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The Artificial Insemination in Sheep of Farmer Flocks Using Semen of Awassi Rams

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The aim of the present study was to inseminate ewes of Malpura breed in the farmer flocks of semi-arid region with semen of exotic Awassi rams to bring about improvement in their productivity.

For the A.I.trial 3 flocks were selected from cluster of villages near Avikanagar in Tonk District of Rajasthan. Ejaculates of adult Awassi rams were collected daily in quick succession by using artificial vagina and examined for its quality. Samples having thick consistency, rapid wave motion (+5) and 90% motility were diluted @ 1:1 with egg yolk McIlvaine glucose (EYMG) diluent (Mathur et al., 1993), filled into round bottom screw cap glass tubes and packed in semen shipper containing ice. The average sperm concentration on the first and last day of collection was 2868 and 3027 million spermatozoa per ml, respectively. A.I. was done with 0.1 ml of freshly diluted semen in ewes detected in oestrus by single per-os insemination for one oestrous cycle in monsoon season of 1995.

The incidence of oestrus was 37% in adult, 26% in 4 teeth, 20% in 2 teeth and 17% in aged (above 6 years) ewes. The duration of oestrus was 24 hours in 73%, 36 hours in 24% and 48 hours in 3% ewes. These results are comparable with the values recorded by Sahni and Roy (1967) on farm flocks of Bikaneri ewes under semi-arid conditions. The overall lambing rate of about 68% achieved in the present study could be considered satisfactory because A.I. was done only in one oestrous cycle. This lambing rate can very well be compared with earlier A.I. trail conducted in ewes of farm flocks at this institute (Tiwari *et al.*, 1973).

Best results with ewes in natural oestrus have been reported when the insemination dose contains 100 to 125 million motile spermatozoa (Salamon, 1962). The insemination dose used in this study meets the requirement of adequate number of motile spermatozoa. Better lambing rates could have been achieved by continuing the A.I. programme during second and subsequent oestrous cycles.

REFERENCES

Mathur, A.K., Srivastava, R.S.and Joshi,Anil (1993). Effect of cortisol incorporation on preservability and metabolism of ram spermatozoa extended in EYMG extender. Indian J.Anim.Reprod., *14 :* 39-40.

Sahni, K.L. and Roy, A(1967). A study on sexual activity of Bikaneri sheep and conception rate through artificial imsemination. Indian J.Vet.Sci., *37 :* 327-334.

Salamon, S. (1962). Studies on he artificial insemination of Merino sheep. II. Effect of frequent ejaculation on semen characteristics and fertilizing capacity. Aust.J.Agric.Res., *13 :* 1137-1150.

Tiwari, S.B.,Honmode,J.,Singh, V.K.,Singh,L.B.and Sahni,K.L.(1973). Some observations on oestrous activity and artificial insemination of different breeds of sheep (exotic, native and half-breeds) under semi-arid conditions. Indian J.Anim.Sci., *43 :* 519-524.

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The Genesis of Veterinary Gynaecology in India

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Though the country had some of the finest breeds of Livestock for milk as well as draught purposes there were no efforts to conserve and improve the genetic resources before the dawn of Independence. However there was surge of interest in post Independent India to improve the productivity of Livestock for milk, meat and draught. The first step in this direction was the launching of Key Village Scheme in the year 1952. The broad objective of this scheme was to use the technique of artificial insemination as a tool for the upgrading and improving the Livestock particularly Cattle in compact areas. As the academic programmes and research in ;the field of Veterinary Science and Animal Husbandry were not fully developed in the country the assistance of FAO was requistioned for providing expertise from other developed countries to reorganise veterinary education and research activities.

The Animal Gynaecology, semen prodcution and Artificial Insemination disciplines had by then progressed in several Scandinavian countries. The FAO provided the services of 3 Swedish experts consisting of Prof.Nils Lagerlof and Dr.Palsson and Dr.Settergren to prepare a blue print for Livestock development in India. The above team was also entrusted to investigate the incidence and causes of infertility in cattle.

The team conducted extensive survey of veterinary education and studied the functioning of A.I. services. in the country in 1953 and prepared a blue print for development of Livestock.

The team of experts reported that education and training of field staff were the most important factors in developing Artificial Insemination programme and in tackling the most common reproductive disorders. The committee stressed the need to initiate research on sexually transmitted Livestock diseases and disorders. The need for the establishment of a centralised semen collection centre to prevent sexually transmitted livestock diseases was also an important recommendation the committee made in its report.

The team also suggested establishment of central sterility station at I.V.R.I. Izatnagar and regional centres at Veterinary colleges in Bombay, Calcutta, Madras, Mathura, Hissar and Patna. Later these centres developed the nucleas for the expansion of Artificial Insemination activities on scientific lines.

Dr.Palsson conducted a six months tranining course on Artificial Insemination at I.V.R.I. Dr. Lundgren another Swedish expert conducted short training courses on A.I. in the states of Bombay, Mysore, Madras, Hyderabad, Tranvancore, Orissa, Bihar and Uttar pradesh.

The expert committee also recommended that the academic programmes in Veterinary colleges should be reorganised to teach and conduct research in Gynaecology obstetrics and Artificial Insemination under one roof in a common department.

The expert committee recommended establishment of exclusive chairs of obstetrics and gynaecology in Veterinary colleges in India. They have also suggested establishment of ambulatory clinics in all

Veterinary colleges to provide practical training to students and to enable access to veterinary health care and Artificial Insemination services in rural areas.

The FAO and Swedish international development agency (S.I.D.A.) came forward to organise a special advanced post-graduate course in animal reproduction of nine months duration at Royal Veterinary College, Stockolm under the guidance of Prof.Lagerlof. The first course commenced in 1954 and the Veterinarian who had the opportunity to attend the course are Dr.G.B.Singh, Dr.C.R.Sane, Dr.S.N.Luktuke, Dr.S.M.Ishaqe, Dr.K.K.Vyas, Dr.M.P.Johari, Dr. B.K.Basu and Dr. Chellam. On completion of the course the trainees received a diploma of "Fellowship of the Royal Veterinary College", Stockholm.

The curriculam for the above training included lectures, seminars, group discussions and practical training in Gynaecology, Obstetrics, Artificial Insemination and bull investigations. The practical training was imparted in slaughter houses and bull stations under the guidance of senior veterinarians.

This training was most sought after and Government of India deputed 80 more veterinarians to undergo this course over a period of 25 years. Another significant aspect of this scheme was that almost all professors chairs in India were occupied by veterinarians trained in Sweden, which ensured establishment of high academic standards and uniformity in teaching veterinary gynaecology and obstetrics. Thus the establishment of independent chairs of obstetrics and Gynaecology in every Veterinary college is a landmark in the history of Veterinary Education in India.

Trainees with Dr. Palsson

Trainees with Dr. Lundgren