## DOSE OF NORGESTOMET AND ITS EFFECT ON THE SECRETARY PATTERN OF HORMONES IN KANGEYAM COWS

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#### ABSTRACT

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Twelve Kangeyam cows were synchronised with double injection of PGF, a at 11 days interval. At induced oestrus the cows were divided into groups as 1 and 2 consisting of six cows each. Group 1 and 2 were treated with one norgestoment and two norgestomet implants respectively. The day of implant Insertion was considered as day 0 and the implants were kept in situ for 9 days. In both the groups 25 mg of PGF, α was administered on the day of implant insertion. The concentrations of progesterone, oestradiol and luteinizing hormones were assayed. During the treatment period the progesterone concentration did not show any significant difference between the groups. The luteinizing hormone registered an increasing trend in group 1 and such trend was not observed in group 2. The oestradiol concentration increased upto day 5 of the treatment period in both the groups and then it started to decrease in group 2 and consistently increased in group 1.

Norgestomet, a synthetic progestogen successfully used to synchronize the oestrus in cows, but the fertility is inconsistent

(Rajamahendran and Taylor, 1991; Kojima et al., 1992; Sanchez et al., 1993). It has been suggested that during the treatment period progesterone released from the progestogen preparations (CIDR, PRID, Norgestoment implant) plays a regulatory role on gonadotropin secretion, preparation of endometrium and maintenance of pregnancy. Administration of low dose of progestogen mimics the follicular phase of the oestrous cycle, increases luteinizing hormone pulse frequency and oestradiol secretion which in turn results in low conception rate, (Roberson et al., 1989; Sirois and Fortune 1990; Sanchez et al., 1995). Hence, the objective of the present study is to find out the dose of norgestoment which mimic the midluteal phase of the oestrous cycle in Kangeyam cows.

## MATERIALS AND METHODS

Twelve Kangeyam cows (2-4 years of age, 250-300 kg of b. wt.) exhibiting regular oestrous cycle at the interval of 19-21 days were used in his study. The oestrus was synchronized with double injection of  $PGF_{2}\alpha$  at 11 days apart. The induced oestrus was

observed and the cows were randomly assigned into groups 1 and 2 consisting of six cows each. Then the group 1 and 2 were treated with one norgestomet and two norgestomet implants respectively. The day of implant insertion was considered as. day 0 and the implants were kept in situ for 9 days. In both the groups 25 mg. of PGF,  $\alpha$  was administered on the day of implant insertion to remove the endogenous progesterone. Blood samples were collected at 24 hours interval from the day of implant insertion to the day of removal in both the groups. Plasma was separated and stored at 20° C until the assay of progesterone, oestradiol and luteinizing hormone. Concentrations of progesterone and oestradiol were assayed by solid-phase radio immunoassay (coat - a - count kit, Diagnostic Product Corporation, Losangels, USA.) and the concentration of luteinizing hormone was assayed by liquid phase double antibody radio immunossay as per the procedure described by (Sufi et al., 1986). Purified bovine LH (USDAbLH-B-6) and LH antiserum (NIDDKanti-oLH-1, AFP-192279) were used. The sensitivity of this assay was 0.14 mg/ml, and intra-and interassay coefficient variations were 4.90 to 8.48 and 5.4 to 9.9 per cent, respectively. The radioactivity was counted in 125 I (Riastar, Packard 5405, USA) gamma counter. All collected data were statistically analysed by student's `t' test (Snedecor and Cochran, 1989).

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#### **RESULTS AND DISCUSSION**

Hormonal profile during different days of treatment period in group 1 and 2 are depicted in Fig 1 and 2 respectively.

The progesterone concentration on the day of implant insertion was 1.60 ± 0.12 ng/ml in group 1 and 1.58 ± 0.14 ng/ml in group 2. After the administration of PGF, α, the progesterone concentration started to decrease and reach the concentration of 0.5 ng/ml with in 24 - 48 hours, and then the same level was maintained throughout the treatment period in both the groups. It suggests that administration of the PGF, a caused lysis of existing corpus lutem in both groups. Peters et al. (1984) stated that in norgestoment treated cows the progesterone (norgestomet) concentration in the peripheral circulation was non detectable by radio immunoassay since the synthetic progestogen (norgestoment) did not react with labelled progesterone antibody. Hence, it is inferred from the study that the low level progesterone recorded in norgestoment treated cows might indicate only the endogenous progesterone present in the circulation.

The luteinizing hormone concentration on the day of implant insertion was  $0.58 \pm 0.02$  ng/ml in group 1 and  $0.56 \pm 0.03$  ng/ml in groups 2. During the treatment period,

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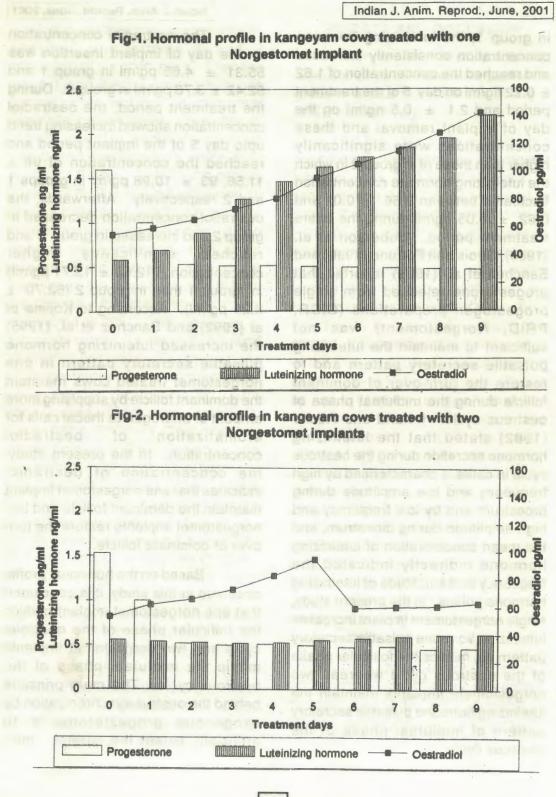
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in group 1, the luteinizing hormone concentration consistently increased and reached the concentration of 1.62 ± 0.22 ng/ml on day 5 of the treatment period and 2.1 ± 0.5 ng/ml on the day of implant removal and these concentrations were significantly higher than those of in group 2 in which the luteinizing hormone concentration fluctuated between 0.56 ± 0.02 and 0.62 ± 0.05 ng/ml during the entire treatment period. Roberson et al. (1989), Sirois and Fortune (1990) and Sanchez et al. (1995) reported that progesterone released from single progestogen preparations (CIDR, PRID, Norgestoment) was not sufficient to maintain the luteinizing pulsatile secretory pattern and to restore the turn over of dominant follicle during the midluteal phase of oestrous cycle. Ireland and Roche (1982) stated that the luteinizing hormone secretion during the oestrous cycle in cattle is characterised by high frequency and low amplitude during proestrum and by low frequency and high amplitude during dioestrum, and the mean concentration of luteinizing hormone indirectly indicated the frequency and amplitude of luteinizing hormone pulses. In the present study, single norgestoment implant increases luteinizing hormone pulsatile secretory pattern i.e. mimics the follicular phase of the oestrous cycle whereas two norgestoment implants maintain the luteinizing hormone pulsatile secretory pattern of midluteal phase of the oestrous cycle.

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The oestradiol concentration on the day of implant insertion was 55.31 ± 4.65 pg/ml in group 1 and 52.42 ± 3.78 pg/ml in group 2. During the treatment period, the oestradiol concentration showed increasing trend upto day 5 of the implant period and reached the concentration of 96 ± 11.56, 93 ± 10.98 pg/ml in groups 1 and 2 respectively. Afterwards, the oestratiol concentration decreased in group 2 and increased in group 1 and reached significantly higher concentration (142.63 ± 13.71 pg/ml) in group 1 than in group 2 (62.70 ± 8.21 pg/ml). According to Kojima et al (1992) and Sanchez et al. (1995) the increased luteinizing hormone pulsatile secretory pattern in one norgestomet treated cows maintain the dominant follicle by supplying more amount of androgen to thecal cells for aromatization of oestradiol concentration. In the present study, the concentration of oestradiol indicates that one norgestomet implant maintain the dominant follicle and two norgestomet implants restore the turn over of dominate follicle.

Based on the hormonal profile observed in this study, it is concluded that one norgestomet implant mimics the follicular phase of the oestrous cycle and two norgestomet implants mimic the midluteal phase of the oestrous cycle. The main principle behind the oestrus synchronization by exogenous progesterone is to artificially extent the normal (mid)



luteal phase of oestrus cycle. So the doses of progestogen that mimic hormonal profile of midluteal phase of oestrous cycle is essential to increase the fertility. Hence, two norgestoment

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implant can be used as physiological replacement of corpus luteum in oestrus synchronization programme in cows.

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## RELATIONSHIP OF SERUM PROGESTERONE CONCENTRATION WITH NUMBER OF OVULATIONS, QUANTITY AND QUALITY OF EMBRYOS IN SUPER OVULATED CROSSBRED CATTLE\*

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#### ABSTRACT

A study was undertaken to relate the super ovulatory response, recovery and quality of embryo at different stages of the cycle with the circulatory progesterone hormone in cross-bred cattle. The concentration of progesterone at initiation of superovulation treatment was correlated (r = 0.34) with subsequent ovulation rate, quantity and quality of embryos. However, progesterone concentration on the day of flushing was significantly correlated with ovulation rate and transferable embryos in FSH treated cows. Ovulation, embryo recovery and transferable embryos were highly related to luteal function at initiation of superovulatory treatment and post superovulatory treatment.

Lack of reliability and variability of ovarian response to gonadotropin treatment poses a major

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limitation towards successful embryo transfer programmes. Moonniaux et. al., (1983) and Donaldson (1985) opined that progesterone concentration in peripheral blood at the time of embryo recovery could be used to indirectly express ovulation rate. Goto et.al., (1987) reported that progesterone concentration on the first treatment day was related to ovarian response and embryo quality. On the contrary, several workers failed to find relationship between the progesterone concentration on the first treatment day and ovarian response and quality of embryos. An attempt has been made to study correlation of serum progesterone at different stages of the cycle with ovulation rate, quantity of embryos in crossbred cattle.

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## MATERIALS AND METHODS

Twelve lactating cows (Jersey x Sindhi) on average 110 days post partum, located in a Farm owned by Tamil Nadu Veterinary and Animal Sciences University were equally divided into two groups. They were superovulated either with Pregnant Mare Serum Gonadotropin (PMSG) or Follicle stimulating hormone (FSH). The gonadotropin treatment was initiated during mid luteal phase of the

## cycle. PMSG (2000 I.U. Folligon, Intervet International, Holland) was injected as single intramuscular injection. However, FSH treated cows received eight intramuscular injection of FSH (Folltropin, Vetrepharm, Canada) given twice daily for four days at doses of 3.6, 2.7, 1.8 and 0.9 mg (Total 18 mg equivalent to 400 NIH-FSH-PI) FSH per injection per day, respectively. Luteolysis was induced by injecting Dinoprost (25 mg) at 48 and 60 hours after initiation of gonadotropin treatment.

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All cows were artificially inseminated with frozen thawed semen at 48,56 and 72 hours after the PG injection. Flushing for embryos was done on day 7 (day of onset of oestrus = day 0) non-surgically, after determining the number of corpora lutea, using flushing catheter (German Rusch 18 gauge). Embryos were examined, counted, graded on a scale of 1 to 5 as per the method described by Goulding et al., (1991). Unfertilised eggs were not included in this grade 1 to 5 classification system.

Blood samples were collected from the animals between 7.30 to 8.30 A.M. by jugular veinpuncture two days prior to gonadotropin treatment, on the day of prostaglandin treatment, on the day of superovulatory oestrum, third day after superovulatory heat and on the day of embryo collection. After centrifugation of the clotted blood at 3000 rpm for 10 minutes, the serum was harvested and stored at - 20 C until assayed for progesterone by Radio immune assay as described by

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Sarma et.al., (1987). The sensitivity of assay was 5 pg/ml in the antisera used. Cross reactivity was less than 1 per cent with related steroids, Inter assay and intra assay variations were 11 per cent and 13 per cent, respectively.

Correlation of concentration of progesterone at pre-treatment, at oestrus, at flushing with ovulation rate, total recovered embryos and viable embryos were estimated. The correlation values tested for significance using standard table (Snedecor and Cochran, 1968).

## **RESULTS AND DISCUSSION**

Among PMSG treated animals two cows had a pre-treatment progesterone value of 2.5 ng/ml and had more than six corpora lutea on day 7 of superovulatory oestrus. Two cows with progesterone value of 2.2 ng/ml had 9 and 3 corpora lutea, respectively. One cow had the pretreatment progesterone level of 2.0 ng/ ml and found to have good response supervulation in by having corporalutea. The cow with pretreatment progesterone of 0.38 ng/ml did not respond well to ovulation (4 corporalutea)

Among FSH treated animals, three animals had a pre-treatment progesterone value of 2.0, 2.4 and 2.5 ng/ml, respectively and showed a good response to superovulation. All these animals had more than eight corporalutea on the day of flushing. Two cows had a similar pre-treatment progesterone level of 4.5 ng/ml, one

cow did not respond well (4 corpovalutea) whereas another cow have a good response in superovulation by having eleven corporalutea. Cow with progesterone level of 1.6 ng/ml showed five ovulation.

The concentration of progesterone at initiation of superovulation positively correlated but not significantly in PMSG treated animals and no such positive correlation was observed in FSH treated animals with subsequent ovulation rate, number of recovered and transferable embryos. Yadav et.al., (1986) observed similar finding while comparing the relationship of progesterone with superovulation responses with FSH and PMSG treatment and opined that it may be possible that some of the cows in the PMSG groups had a good potential for follicular development and as a result had well developed corpus luteum.

The progesterone concentration oestrus in at superovulated cows ranged from 0.06 to 0.55 ng/ml and 0.34 to 0.50 ng/ml in PMSG and FSH treated animals. respectively. The results agree with earlier report of Saumande and Batra (1985) which documented a positive correlation between plasma progesterone on day '0' with ovulation rate, number of recovered and transferable embryos. The concentration of progesterone on the day of flushing (day 7), ranged from 4.0 to > 10.0 ng/ml, increased with an increase in the number of corpus

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luteum in PMSG treated animals while in FSH treated animals, the concentration of progesterone on the day of flushing, ranged from 0.4 to > 10.0 ng/ml. Four cows had highest circulating level of serum progesterone on the day of flushing which was corresponding with highest number of ovulations (8-11). In contrast two cows had maximum progesterone concentration of 4.5 and 0.4 ng/ml with five and four ovulations, respectively. The maximum level of progesterone in cows superovulated with PMSG were showing positive correlation though not significantly while FSH treated animals were showing significant correlation with number of ovulation and viable embryos (r = 0.90)and positively correlated, but not significantly with number of recovered embryos. Appavu and Holtz (1992) opined that the relationship between corporalutea and progesterone may help to distinguish between good and poorly responded superovulated animals. Some of the authors, however, did not find any such relationship (Rajamahendran et. al., 1976, Sreenam and Goshy 1977).

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## COMPARATIVE EFFICACY OF DIFFERENT DRUGS ON SUPEROVULATION AND EMBRYONIC PROFILE IN COWS

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#### ABSTRACT

Comparative ovarian response and production of embryos in 27 lactating cattle (Jersey: Jersey X Red Sindhi) superovualated with Super-OV (n=6), Super-OV + Buserrelin (n=6), Follotropin-V (n=9) and eCG (n=6) have been reported. While the highest number of ovulations were recorded using Folltropin-V, the number of transferable embryos was highest using Super-OV.

For superovulation different workers have tried different doses of variety of drugs either containing FSH or eCG. The results in terms of number of ovulations and embryos recovered per donor have been variable on account of number of factors (Elsden et al., 1978). This study presents the ovarian response and embryo recovery with FSH (Super-OV, Super-OV + Buserelin and Folltropin-V) and eCG (Folligon in lactating dairy cows.

## MATERIALS AND METHODS

In all 27 lactating cows (Jersey; Jersey X Red Sindhi) of varying reproductive parity were

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selected from the University Dairy Farm. All the animals were cyclic, free of any reproductive problem and maintained under standard feeding and management conditions. Depending on the type of treatment the cows were divided into following groups : G

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**Group I (n=6):** The animals were administered (I/M) 75 mg NIH units of Super-OV (Ausa International, Canada) in six equal divided doses at 12 hourly intervals. Twenty five mg of PGF<sub>2</sub> $\alpha$  (Lutalyse; Unichem, India) was administered (I/M) along with fifth injection of Super-OV. The treatment was initiated on day 10 after the observed estrus.

**Group II (n=6):** In addition to the treatment mentioned for Group I, 0.0021 mg of Buserelin, a GnRH analogue (Receptal, Hoechst India Ltd.) was administered (I/M) at first A.I.

**Group III (n=9):** A total dose of 400 mg NIH units of Folltropin - V (Vetrepharm Inc., Canada) was administered (I/M) in 8 equal divided doses at 12 hourly intervals between day 10-13 after observed estrus.  $PGF_2\alpha$  (25 mg of Lutalyse) was administered (I/M) along with 6<sup>th</sup> injection of Folltropin-V.

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**Group IV (n=6):** A single injection of 2500 I.U. of eCG (Folligon; Intervet, Holland) was administered (I/M) after day 10 of observed estrus followed by 25 mg of PGF<sub>2</sub> $\alpha$  48 hours later.

Estrus was detected in donors by visual and clinico-gynaecological examination at 6-10 hourly intervals beginning with 18 hours following  $PGF_2\alpha$  injection. The animals in estrus were inseminated thrice with double straw of frozen semen at 12 hour interval.

Superovulatory response was recorded by rectal palpation of ovaries by counting number of corpora lutea and follicles on the day before flushing of embryos. Embryos were collected on day 5/6 after last A.I. using 2 way Rusch / Folley's cathetar. Dulbeccos Phosphate Buffer Saline supplemented with 1% BSA fraction V was used as flushing medium. The collected media was filtered and embryos examined and classified under zoom stereo microscope (Lindner and Wright, 1983). Student's t-test used for statistical analysis (Snedecor and Cochran, 1967)

## RESULTS AND DISCUSSION

Ovarian response and embryo production with different drugs used in cows is presented in Table

Out of 27 a total of 20 (74.07%) cows responded to superovulatory treatments. The percent of animals responding with Super-OV, Super-OV + Buserelin, Folltropin-V and eCG were 50, 50, 100

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and 83.3 respectively. Khurana (1999) has reported 44.4% cows responding with Super-OV. Ansari et al. (1998) have reported 71.4% and 87.5% crossbred cows responding to Folltropin-V and eCG, respectively.

Superovulatory response with Folltropin-V was 12.33 ± 0.49, which was significantly higher than other treatment groups. Compared to the present findings with Folltropin-V in cows, decreased ovulation rates of 7.6 ± 1.46 (Ansari et al., 1998), 7.6 ± 1.1 (Arora et al., 1996) and 9.33 ± 0.52 (Murugavel et al., 1999) have been reported. Khurana (1999) using Super-OV has reported a higher mean ovulation rate of 8.42 ± 1.9 in crossbred cows. Using eCG almost similar ovulation rate of  $6.6 \pm 1.2$  and  $6.4 \pm 1.9$  have been reported by Arora et al. (1996) and Balakrishnan et al. (1994), respectively. In this study using Buserelin with Super-OV the mean ovulation rate was less than Super-OV alone, though the difference was statistically non-significant (P < 0.05). Our findings are supported by Khanna et al. (1994). However Ansari et al. (1998) have reported increased ovulation rates after using Buserelin with different gonadotrophins.

Large unovulatory follicles (4.66  $\pm$  1.4) were present only with eCG. Almost similar findings of 5.00  $\pm$  0.31 and 5.60  $\pm$  0.5 unovulatory follicles have been recorded after using eCG by Ansari et al. (1998) and Kharche et al. (1993), respectively. Shanker et al. (1998) and Murugavel

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TABLE : Ovarian response, embryo production and Embryo Quality using Different Supervulation Inducing Drugs in Cows

Parameter	Super - OV	Super OV + Buserellin	Folltrophin - V	eCG	
Donors resonded (percent) $\geq$ 3 C.L.	3/6 (50%)	3/6(50%)	9/9(100%)	5/6 (83.3%)	
Corpus Luteum (C.L.)	6.33 ± 2.11	4.66 ± 1.98	12.33 ± 0.49	5.33 ± 1.21	
Unovulatory follicles (F)	Nil	Nil	Nil	4.66 ± 1.4	
Total ovarian response (C.L. + F)	6.33 ± 2.11	4.66 ± 1.98	12.33 ± 0.49	10.0 ± 0.54	
Embryo production	amisan	sutton of clar	nine ant on	GP glained	
Donars flushed	3	3	9	5	
Total number of ovulations	38	28	Ann 111 Ann	32	
Donora yiclding eggs	84.1 3	3	7	3	
Total embryos recovered	9	4	26	8	
Recovery rate (%)	23.68	14.68	23.42	25.0	
Mean / Donor	3.33 ± 0.98	1.33 ± 0.27	2.88 ± 0.85	1.6 ± 1.0	
Embryo quality	Subat-0	form flushing	e yabadi /u	nd follides	
Good	5/9	1/4	15/26	2/8	
Fair	3/9	2/4	7/26	4/8	
Poor	1/9	1/4	4/26	2/8	
Transferable embryos	10.10	Balla S.	Buttan	BIBRIGHON	
Mean/Donor	2.6	1,0	2.4	1.2	
Percent and a dimension	88.8	75.0	84.6	75.0	

et al. (1990) have recorded unovulatory follicles using Folltropin-V. The problem of unovulatory follicles with ECG is because of prolonged follicular development resulting from long half-life of eCG, variable LH content in different batches of drug, pre-ovulatory leutinisation of follicles and perturbed endocrine profiles (Vasishta, 1996).

Embryos could be recovered from all the animals that responded to Super-OV and Super-OV + Buserelin treatment, while 2 animals each from Folltropin - V (22.22%) and eCG (40%)

did not yield any embryos Khanna et al. (1994) reported that approximately 1/3rd of the donors do not produce any embrvo. The mean number of embryos per donor was highest in animals treated with Super-OV (3.33 ± 0.98) bud did not differ significantly from others. Using Super-OV, Khurana (1999) has reported mean embryo recovery rate of 3.2. With 20 mg of Folltropin-V the mean embryo recovery was 4.06 ± 4.2 (Shankar et al., 1998). With eCG Balakrishnan et al. (1994) have reported 5.8 ± 1.8 embryos per donor, whereas with 2000

I.U of eCG  $3.0 \pm 0.9$  (Ansari et al., 1998) and  $4.83 \pm 1.6$  (Arora et al., 1996) embryos per donor have been reported.

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Highest mean percent of transferable embryos per donor was 88.8 with Super-OV followed by 84.6 with Folltropin-V, 75 with eCG and with Super-OV + Buserelin. Khurana (1999)has reported 56 transferable embryos with Super-OV. Using Folltropin-V as a constant dose schedule Arora et al. (1996) have reported 1.83 transferable embryos which simulated to our findings, but the percentage of transferable embryos

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was relatively less (65.6%), however a similar percentage (60%) but relatively higher transferable embryos (4.33) have been reported by Murugavel et al. (1999) after using a similar dose of Folltropin-V in a tapering dose schedule. Another report by Shanker et al. (1998) has shown 2.06 transferable embryos per donor using 20 mg of Folltropin-V. Using eCG Balakrishnan et al. (1994) recovered 4.6 embryos per donor as transferable, whereas with 2000 I.U of eCG Arora et al. (1998) and Singh et. al. (1998) recovered, 75,77% and 20.6% transferable embryos per donor.

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## SYNCHRONIZATION OF ESTRUM BY SINGLE AND DOUBLE DOSE OF PROSTAGLANDIN $F_2\alpha$ in cross bred heifers in SUPEROVULATION PROTOCOLS

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#### ABSTRACT

Estrus was synchronized by the administration of 25 mg each of prostaglandin F2 $\alpha$  (PGF, $\alpha$ ) in 10 cross bred beifers in two doses at 12 days Estrus symptoms and interval. observation as per rectal palpation were recorded at each estrus following administration of each dose of PGF<sub> $\alpha$ </sub>. The mean interval between treatment and appearance of estrum following administration of PGF<sub>2</sub> $\alpha$  was 64.00 ± 10.22 and 52.8 ± 4.07 hours respectively. The percentage of reactors for the first and second dose of PGF,  $\alpha$  was 60.00 and 100 percent respectively. The prostaglandins were effective in inducing luteolysis of previous cycles in 70.00% Vs 100.00% and the follicular development and CL formation following their ovulation was 60.00% Vs 90.00% and 50.00% Vs 100% in single and double injection groups respectively. Administration of prostaglandin in cattle in two divided doses

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with interval of 12 days rather than in a single dose, thus, helps in inducing earlier and more intense heat. Development of follicles and CL formation are found in a greater number of cases.

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In superovulatory protocols, synchronization of estrum is essential to induce a good response to FSH treatment and for synchronizing cycles between the donor and recipient animals for proper egg implantation. The CL can be knocked down by the administration of prostaglandin  $F_{\alpha}a$ . The regression of CL occurs within 24-72 hours after administering PGF.a. Administration of double dose of PGF, $\alpha$  at 10-14 days apart. synchronized estrus in higher percentage donors and recipients (Odde, 1990, Reddy et.al. 1992). Goulding et.al., (1990) reported that when double dose of PGF,  $\alpha$  is used for estrus synchronization, the estrus was usually observed within 48 hours and in some protracted cases from 72 to 96 hours.

## MATERIALS AND METHODS

Estrus was synchronized in

10 cycling crossbred heifers under superovulatory protocols. All the animals were specially prepared by maintaining them on balanced ration consisting of greens, roughage, concentrates and mineral vitamin mixture for at least 60 days prior to hormonal treatment. All the 10 animals were synchronized in estrus by administering two doses of 25 mg Dinofertin each of (Alved Pharmeceuticals, Chennai) at 12 days interval. The symptoms of estrum and observations on per-rectal palpation were recorded between 36 hours to 96 hours following administration of PGF, $\alpha$  at each time (i.e., after first -Group A and after second dose Group B), The results were analysed by paired `t' test.

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#### **RESULTS AND DISCUSSION**

Following administration of PGF<sub>2</sub> $\alpha$  the induction of estrus varies significantly (p < 0.05) between the single (Group A) and double (Group B) dose of PGF<sub>2</sub> $\alpha$  with respect to the number of animals reacted, interval between the administration of PGF<sub>2</sub> $\alpha$  and appearance of estrus. While four animals failed to show any response to the first dose of PGF<sub>2</sub> $\alpha$ , all the 10 animals, responded to the second dose of the drug.

The mean interval between administration of  $PGF_2\alpha$  and appearance of estrum following the single and double dose of  $PGF_2\alpha$  were 64.00 ± 10.22 and 52.80 ± 4.07 hours respectively. These observations are

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similar to those reported by Chauhan et. al., (1994) who reported 48.78  $\pm$ 2.45 hours of the interval between treatment and appearance of estrus in superovulated cows and Jacob et. al (1995) who reported 58.95 hours of similar observation in crossbred cows treated with 25 mg. PGF<sub>2</sub>a having postpartum clinical endometritis.

The percentage of reactors for single and double dose of PGF, $\alpha$ were 60.00 and 100.00 respectively which is marginally lower when compared to those reported by Chauhan et.al., (1994) for a single dose of PGF, $\alpha$  (80%) in Jersey, Kankrej crossbred cows. However, the double does PG treatment in the present study registered higher percentage of reactors. The present results are in agreement with those observed by Young (1989), Odde (1990) and Whittier et.al., (1991) who noted that comparatively higher percentage of donor and recipients responding to the second dose of PGF, $\alpha$  and reduced interval between  $PGF_{\alpha}$  administration and appearance of estrum. This has been attributed to the timing of luteal receptivity to the second dose of PGF,a.

Further, the intensity of estrus symptoms which was more prominent following second dose of  $PGF_{2}\alpha$  might have been the effect of promotion of follicular development following luteolysis as reported by Hansel and Convey (1983).

Per rectal palpation of donors on day - 0 revealed 70% and 100% luteolysis of the CL of previous cycles in group A and B respectively as evidenced by the absence of functional luteal structures. The above results for first dose treatment was less than those observed by Mane et.al., (1992), who reported 100% luteolysis following signal dose of PGF, $\alpha$ , but similar results were obtained in the present studies following second dose of PGF, $\alpha$ . The follicular development during the same period was 60% and 90.5% in group A and B respectively and the CL formation following their ovulation as observed on day seven

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of the cycle was 50% in group A and 100% in group B animals. From the above observations it may be concluded that in cattle the administration of prostagladin in divided doses with interval of 12 days rather than in a single dose helps in obtaining early expression of estrum, follicle development and CL formation in greater number of cases.

## ACKNOWLEDGEMENT.

The authors wish to record the assistance provided by M/s. Alved Pharmaceuticals Ltd., Madras for providing part of the requirement of Prostagladin  $F_2\alpha$  (Dinofertin) as gift for the present experiment.

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## RESPONSE OF MURRAH BUFFALOES TO DIFFERENT SUPEROVULATORY DRUGS.

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## ABSTRACT

An investigation was carried out to determine the efficacy of four superovulatory drugs i.e. Folltropin (Vetrepharma, Canada), FSH-P (Schering, USA) Ovagen (Immuno Chem. Products Ltd. Auckland NZ) and Folligon (Intervet, Holland). These gonadotropins were administered to Murrah buffaloes in mid luteal phase. The index for inducing the response of Murrah buffaloes to these superovulatory drugs was presence of corpora lutea and number of embryos recovered. It was found that the response was higher in Folltropin  $(5.5 \pm 1.0 \text{ and } 2.75)$  $\pm$  1.49) as compared to all the other three groups i.e. FSH-P (3.17 ± 0.38 and 0.5 ± 0.14), Ovagen  $(2.5 \pm 0.92 \text{ and } 0.85 \pm 0.40)$ and Folligon  $(1.0 \pm 0.7 \text{ and } 0.0 \pm 0.0)$ 

A number of drugs with different protocols have been used to superovulate buffaloes. The object of the present study is to compare the efficiency of four different superovulatory drugs (Folltropin, FSH-P, Ovagen and Folligon) in buffaloes.

## MATERIALS AND METHODS

Thirty four buffaloes showing normal reproductive cyclicity and having no palpable abnormalities were selected as experimental animals and were divided into four groups. Animals were maintained under standard feeding and managerial practice. Buffaloes were subjected to superovulation using four different drugs i.e. Folltropin, FSH-P, Ovagen, and Folligon on day 11th of oestrous cycle. Folltropin was used in four animals with 50 mg. divided into eight doses twice for four days similarly 46 mg, of FSH-P divided into eight decreasing does was used for four days on eighteen animals. Ovagen was used in seven animals with a total dose of 10 mg. @ 1.25 mg. twice for four days while Folligon single dose of 2500 I.U. was used on five animals. PGF<sub> $\alpha$ </sub> (Lutalys 25 mg.) was given on day 3 of starting superovulatory treatment to each animal under experiment. The animals were then closely monitored for heat detection. The buffaloes expressing heat were

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inseminated twice with frozen semen. Embryos were collected by standard non-surgical technique on day 7 post breeding in D-PBS medium containing 0.1 per cent BSA fraction V in flushing medium using Folley's catheter Embryos were collected by running the flushing medium through filter. All the recovered embryos/eggs after their location were transferred into holding medium (D-PSB ± 0.4% BSA) and were subjected to classification as viable, degenerated and unfertilised ova. Statistical analysis of the data was done as per Snedecor and Cochran (1971). Iste mediately of our

## **RESULTS AND DISCUSSION**

It was found that the response was higher in Folltropin ( $5.5 \pm 1.0$  and 2.75  $\pm 1.49$ ) as compared to all the other three groups i.e. FSH – P (3.17

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± 0.38 and 0.5 ± 0.14), Ovagen (2.5 ± 0.92 and 0.85 ± 0.40) and Folligon (1.0 ± 0.77 and 0.0 ± 0.0) respectively for corpora lutea and embryo recovery. The data showed that total response was higher in Folltropin. The findings of Misra et.al (1989) showed a better superovulatory response using the same drug which might be because of purer form of drug available in this commercially available preparation as compared to other products used. Use of Folligon had resulted in nonovulatory follicles and ovaries appeared as if they were golf balls leading to poor embryo recovery rate possibly due to prolonged half life time of this drug. down bre setul anarroo

The embryo recovery rate in this experiment was in agreement to the work reported to Drost et.al., (1985): Jain, et.al., (1990).

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## COMPARATIVE EFFICACY OF ASPIRATION AND DISSECTION TECHNIQUES ON THE RECOVERY OF OOCYTES FROM GOAT OVARIES

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#### ABSTRACT

A total of 200 goat ovaries with intact reproductive tract were collected immediately after slaughter in a thermosflask containing warm (35° C) physiological saline. The ovaries of different animals were assigned to aspiration and dissection techniques for recovery of oocytes alternately. In aspiration and dissection techniques the number of oocytes recovered per ovary was 2.01 and 2.23 respectively and the rate of recovery was 76.72 and 85.11 per cent respectively, the difference being significant (p < 0.05). The recovery of oocytes of A, B, C and D categories was 39.69, 9.16, 10.69 and 17.18 per cent respectively for aspiration technique and 54.58, 16.03, 8.02 and 6.49 per cent respectively for dissection technique. The recovery of oocytes of A category and A + B categories was significantly (p < 0.05). higher in dissection technique.

A part of the M.V.Sc.. thesis of first author, Assam Agricultural University, Khanapara Campus.

1 Veterinary Assistant Surgeon, State Vety.. Dispensary, Moranhat, Dist. Sivsagar, Assam.

2 Associate Professor, Department of Physiology. Follicular oocytes could be matured in vitro and used for in vitro fertilization for producing a large number of embryos, (Agrawal, 1992: Suzuki et al: 1992). With this end in view, the present work was undertaken to compare the efficacy of aspiration and dissection techniques for recovery of oocytes from follicles of goat ovaries.

#### MATERIALS AND METHODS

Two hundred goat ovaries along with the reproductive tract were collected immediately after slaughter from private abattoirs of Guwahati and Military butchery, Narengi, Guwahati. The organs were brought to the laboratory in a thermosflask containing warm (35° C) physiological saline solution. In the laboratory, the right and left ovaries were labelled seperately and then washed thoroughly in warm saline solution and the visible follicles measuring 2 to 5 mm in diameter were counted for individual ovary. The ovaries of different animals were assigned to aspiration and dissection techniques alternately for recovery of oocytes.

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Aspiration Technique : Dulbecco's phosphate buffered saline (PBS) with 10% heat inactivated goat serum (HIGS), 1 ml was taken in a 2 ml disposable syringe attached to a 20 gauze needle. The needle was introduced into the follicle through the ovarian stroma at the edge of the follicle and then the content of the follicle was aspirated out and placed in an embryo collection dish containing PBS with 10% HIGS.

Dissection Technique : The visible follicles measuring 2 to 5 mm in diameter were dissected out from the The stromal tissues were ovaries. then removed from the follicles as far as possible. The follicles were then taken in a petridish containing PBS + 10% HIGS and ruptured by using two pairs of pointed forceps under stereozoom microscope. The oocytes with adhering cumulus complexes were transferred to an embryo collection dish containing PBS + 10% HIGs.

The oocytes after recovery by the two techniques were examined under stereozoom microscope and the number and categories of oocytes for both right and left ovaries were determined. Depending in cumulus cells layer oocytes were categorized as A, B, C and D following the method of Singh and Sarma (1991).

## **RESULTS AND DISCUSSION**

The number of oocytes recovered per ovary (2.12 oocytes) in

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the present study is comparable to the values of Chakravarty et al. (1994) in Assam local goats. But the present value is much lower than that of Agrawal (1992) and Wahi et al. (1992). who recovered 12.4 and 4.4 oocytes per ovary respectively. On the other hand, Mogas et al. (1992) recovered lower number of oocytes (1.72 oocytes) per ovary than that in the present study. The recovery rates of oocytes from the ovarian follicles were 76.72 and 85.11 per cent in aspiration and dissection techniques respectively. The recovery rate of oocytes in aspiration technique in the present study is comparable to that of Maytino et al. (1992). But the oocyte reported recovery rates bv Balasubramanian et al. (1991) in aspiration and dissection techniques were lower than the present values. The above variations in different studies might be due to differences in the functional state of ovaries in different breeds, seasons and age/or in the procedure of recovery.

It was observed that the recovery rate of oocyte was significantly (p < 0.05). higher in dissection technique (2.25) than in aspiration technique (2.10). This is in agreement with the observations of Balasubramanian et al. (1991) and Wahid et al. (1992). On the contrary, Mogas et. al. (1992) obtained much lower recovery rate in dissection technique and Martino et al. (1992) obtained similar recovery rates in both the techniques. The above

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TABLE	:	Incidence of different categories of oocytes recovered from ovarian	
		follicles of goats in two techniques	

Categories		Aspiration technique				Dissection technique						
of	· · · · · ·	t ovary 31) %		ovary 131) %		<b>verali</b> 262) %	~	t ovary 34) %		ovary 128) %	· ·	it ovary 262) %
MoreAstogic	48	36.64	56	42.75	104	39.69	72	53.73	71	55.47	143	54.58
anna Biorra	10	7.63	14	10.69	24	9.16	26	9.16	16	12.5	42	16.03
C	13	9.92	15	11.45	28	10.69	8	5.97	13	10.16	21	8.02
Dan Cont	28	21.37	17	12.98	45	17.18	10	7.46	7	5.47	17	6.49

Figures in parentheses indicate number of ovaries

discrepancy might be due to differences in size of the needle used, size of the ovarian follicles and procedure used for aspiration and dissection techniques. The higher recovery rate of oocyte in dissection technique might be attributed to lower chance of missing oocytes as the dissected and cleaned follicles were ruptured under microscope. The practical difficulty in aspiration of oocyte which are tightly adhered to granulosa cells in smaller follicle might also influence the recovery rate in aspiration techniques.

Out of different categories of oocytes recovered (table) the oocytes of A category was the highest in both aspiration and dissection techniques. The recovery of D category oocyte (free of cumulus cells) was the lowest in dissection techniques but was relatively higher in aspiration technique. This is in agreement with the observations of earlier workers (Mogas et al., 1992; Wahid et.al. 15.32; Chakravarty et al.; 1994). This might be attributed to the fact that aspiration

of the follicular oocytes and subsequent forceful expiration using needle and syringe might cause detachment of the loosely adhered cumulus cells from the oocyte. The recovery rates of oocytes of A and A + B categories were significantly (p < 0.05). higher in dissection technique than in aspiration technique (Table). This supports the findings of earlier workers (Balasubramanian et al., 1991; Mogas et. al.; 1992; Wahid et al., 1992). On the contrary, Martino et al. (1992) did not observe much difference in recovery of these categories of oocytes between aspiration and dissection techniques. It was reported that oocytes that had a good cumulus investment were more suitable for in vitro maturation and in vitro fertilization (Fukui et al. 1988. Agrawal, 1992; Suzuki et al 1992). The higher recovery rate of oocytes with more cumulus cells investment around zona pellucida in dissection techniques in the present study indicated the superiority of this technique over aspiration technique.

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# P.K. BORDOLOI, B.C. SARMAH, D.J. DUTTA AND B.C. DEKA<sup>1</sup>

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## ABSTRACT

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The concentration of macro and mirco minerals in follicular fluid of small (2-3 mm. in dia), medium (>3-5 mm in dia) and large (>5 mm in dia) follicles were studied in a total of 372 numbers of goat ovaries obtained from the abattoir immediately after slaughter. It was found that the concentrations of macrominerel, calcium and magnesium increased significantly in large follicles, but that of phosphorous decreased significantly as the size of the follicle increased. The concentrations of microminerals Zn, Fe, and Cu were significantly higher in medium follicle and lower in large follicle. The concentration of Mn in follicular fluid increased significantly along with increase in size of the follicle.

Existing reports on chemical composition of follicular fluid, particularly in goat are limited. Therefore, the present study was undertaken to estimate the concentrations of macro and micro minerals in follicular fluid in follicles of different sizes in goat.

Professor, Dept. of Gynaecology, Obstetrics and A.I.

## MATERIALS AND METHODS

A total of 372 numbers of goat ovaries were collected from the abattoir immediately after slaughter and brought to the laboratory in a thermo flask. The ovaries were washed thoroughly with tape water and then with deionised distilled water, and subjected to follicle dissection for classification of follicle according to its diameter as described by Dutta et al. (1996). Dissected follicles were classified as small (2-3 mm in dia), medium (3-5 mm in dia) and large (>5 mm in dia). Follicles belonging to same class were placed in a sterile cavity glass slide and ruptured under steriozoom microscope. Follicular fluid was then drawn from the cavity glass slide and subjected to centrifugation (2000 rpm for 15 min.) and supernatant fluid was used for mineral estimation.

For estimation of macro and micro minerals in follicular fluid one ml. of follicular fluid from each class of follicle was added to 9 ml of 10% TCA. The mixture of follicular fluid and TCA was allowed to stand for 15 minutes after proper shaking. The mixture was subjected to centrifugation at 1500 rpm for 15 minutes. The supernatant was collected and minerals (Ca, P, Mg, Zn, Fe, Cu and Mn) were estimated using Atomic Absorption Spectrophotometer (Model GBC 932, Australia). The statistical analysis of the data was made as per Snedecor and Cochran (1968).

## **RESULTS AND DISCUSSION**

The results are presented the table. The concentration of different macro and micro minerals in follicular fluid varied significantly (p < 0.01) between small, medium and large follicles. The concentration of calcium, magnesium and manganese increased significantly (p < 0.01) as the follicular size increased. The concentration of Zinc, Copper and Iron found to be the highest in medium follicles and lowest in large follicle.

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Higher concentration of calcium in large and medium classes of follicle might be associated with proliferation of granulosa and thecal cells and thus indirectly reflected the steroidogenic capabilities of the growing follicles as calcium plays an important role in gonadotropic regulation of ovarian steroidogenesis (Carnegic and Sang, 1984 and Veldhius et.al., 1984).

The mean concentration of phosphorus recorded in different classes of follicle was lower than that reported in bubaline follicular fluid by Murty et.al. (1987). The significant (p < 0.01) variation in phosphorus recorded in different class of follicle reflects the variation in metabolic activity in follicles of different sizes.

news from the cavit	Macro minerals (mg%)			Micro minerals (mg%)				
Classes of follicle	Ca	Р	Mg	Zn	Fe	Cu	Mn	
Small (2-3 mm)	8.88ª	10.49ª	23.27ª	3.49ª	3.40ª	0.62ª	0.28ª	
	±0.07	±0.45	±0.17	±0.11	±0.49	±0.04	±0.01	
Medium (>3-5 mm)	9.26 <sup>b</sup>	9.31 <sup>b</sup>	24.90 <sup>b</sup>	4.60 <sup>b</sup>	4.70 <sup>b</sup>	0.67 <sup>b</sup>	0.31 <sup>b</sup>	
tion wore class o	±0.08	±0.35	±0.36	±0.16	±0.14	±0.01	±0.01	
Large (>5 mm)	10.36°	7.73°	26.52°	2.56°	2.29°	0.18°	0.38°	
stand for 15 minute	±0.01	±0.01	±0.02	±0.01	±0.01	±0.01	±0.01	

TABLE 1 : Concentration of Macro and Micro Minerals (Mean + SE) in follicular fluid of different classes of follicle

Means bearing different superscript differ significantly (P < 0.01) within column.

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Increase in magnesium concentration along with increase in size of the follicle in the present study was in agreement with the findings of Wise (1987). This might be considered as attainment of steroidogenic capability of the growing follicle as it was suggested that follicular magnesium concentration was positively correlated to follicular androgens (Wise, 1987).

The concentration of Zinc and Iron decreased significantly in large follicle as compared to small and medium sized follicles. This was in agreement with the findings of Kaur Indian J. Anim. Reprod., June, 2001

et.al. (1977) in buffalo ovary. Significantly (p < 0.01) higher copper concentration recorded in small and medium follicles than in large follicles in the present study is in agreement with the findings of Kaur et.al. (1997) in buffalo ovaries. This higher concentration of copper in small and medium sized follicle corresponds to oestrogen secreting stage of the follicle. The mangnese concentration steadily increased with advancement of the follicular size. Sikka (1992) suggested that high concentration of manganese in large follicles might be due to involvement of this ion in major energy producing reaction.

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## A STUDY ON THE SUPEROVULATORY RESPONSE AND EMBRYO RECOVERY RATE IN ASSAM LOCAL GOATS

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#### ABSTRACT

Seven cyclic Assam local goats were treated with exogenous hormone regime (750 i.u. PMSG : 5 mg PGF<sub>2</sub> $\alpha$ alpha: 1500 i.u. HCG) for superovulation. The mean time of induction and duration of oestrus was 19.08 ± 1.23 and 33.57 ± 0.67 hours respectively. The mean number of follicles matured and ovulated per animal were 13.43 ± 2.36 and 10.24 ± 2.75 respectively, the rate of ovulation was 72.36 ± 9.40 per cent.

To meet the scarcity of superior female germ plasm, it is necessary to carry out superovulation and embryo transfer. The response of superovulation varies from species to species, individuals and dose and nature of exogenous hormones used. Hence an attempt was made to study the superovulatory response of Assam local goats to exogenous hormones.

#### MATERIALS AND METHODS

Seven apparently healthy

1 Associate Professor, Department of Physiology. Assam local goats aged 2-3 years and kidded at least once were used for the study. The goats were allowed to graze in the morning and evening and provided with concentrate mixture @ 200 g/ animal/ day. in

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The oestrous cycles of the does were observed closely for 3-4 months. The does used in the study were having regular oestrous cycles. On day 6 of oestrous cycle does were injected with PMSG (Folligon) 750 i.u. (i.m.) followed by PGF<sub>a</sub> alpha (Lutalyse) 5 mg (i.m) on day 7 of the oestrous cycle. hCG (Chorulon) at the dose of 1500 i.u. was administered (i.m.) at 6th hour of the induced oestrus (Chakravarty, 1992) The does were closely observed for signs of oestrus by visual inspection as well as by parading a vasectomised buck at an interval of 4 hours between 6 a.m. and 10 p.m. The does were considered to be in oestrus when they showed receptivity to male. Two crossbred bucks were allowed to breed the does at an interval of 6 hours till cessation of oestrus. The time intervals for induction of oestrus from the time of administration of PMSG and of PGF,  $\alpha$  and the duration of

<sup>\*</sup> A part of M.V.Sc. thesis of first author submitted to Assam Agricultural University, Khanapara campus.

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induced oestrus were recorded in hours.

Mid-ventral laparotomy of the superovulated does was done on 6th day post-mating and embryos were collected by utero-tubal flushing using Dulbecco's phosphate buffer saline (PBS) + 10% heat inactivated goat serum (HIGS) as flushing medium. PBS + 20% HIGS was used as holdings medium for embryo. The ovarian response to superovulatory treatment was studied by recording the number of corpus haemorrhagicum and unovulated matured follicles (Graafian). The total number of corpora haemorrhagica and unovulated matured follicles was considered as total number of follicles produced per ovary as a result of exogenous hormone administration.

## **RESULTS AND DISCUSSION**

All the seven goats exhibited oestrus following superovulatory treatment in the present study. This is in agreement with the findings of earlier workers (Agrawal, 1986; Mani and Vadnere, 1989: Bonia, 1992). The mean time intervals for duration of oestrus from administration of PMSG and PGF,  $\alpha$  alpha were 43.03 ± 1.23 and 19.08 ± 1.23 hours respectively. The time of induction of oestrus from administration of PGF, a alpha recorded in the present study is comparable to that recorded by Bonia (1992) and Chakravarty (1992) in Assam local goats. On the contrary, the time of induction of oestrus from

administration of PMSG in the present study is much shorter than that recorded by Agrawal (1986) in Barbari goats and Doijode et al. (1992) in Osmanabadi and crossbred goats treated with progesterone and PMSG. On the other hand, Eiamvitayakorn et.al., (1988) recorded a shorter oestrus induction time in goats treated with FSH and PGF<sub>2</sub> $\alpha$ . The above variations in oestrus induction time might be attributed to the differences in the type of hormones and doses used, time of administration, bread of goats and individual response.

The duration of induced oestrus (33.57 ± 0.67 hr.) following superovulatory treatment recorded in the study is comparable to that of Bonia (1992) and Chakravarty (1992) in Assam local goats treated with PMSG: PGF,  $\alpha$  and Pargaonkar et.al. (1992) in Osmanabadi and crossbred goats treated with progesterone: PMSG. On the other hand, Doijode et al. (1992) recorded shorter duration of induced oestrus in Osmanabadi and crossbred goats treated with progresterone; PMSG. The duration of induced oestrus recorded in the present study was within the range stipulated for normal cyclic goats (Hafez, 1987).

Regarding the superovulatory response and embryo recovery rate in Assam local goats the mean number of follicles in Assam local goats in response to exogenous hormone was found to be  $13.43 \pm 2.36$  per goat. The

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present value is comparable to that of Mani and Vadnere (1989) in goats treated with progesterone: PMSG: hCG. On the contrary, the present value was higher than that in Osmanabadi and crossbred goats treated with progesterone : PMSG and hCG (Pargaonkar et.at., 1992), Black Bengal goats treated with progesterone or PGF<sub>2</sub> $\alpha$  alpha: FSH + LH (Pandey et.al., 1992) and in Pashmina goats treated with FSH – P: PMSG (Mahmood et.al., 1991).

The mean number of ovulation per animal following superovulatory treatment was 10.24 ± 2.75. The present value is comparable to that in Angora goats treated with PMSG: PGF,a alpha (Armstrong and Evans, 1983), and in Black Bengal goats treated with progesterone : PMSG : hCG (Pargaonkar et.al., 1992). On the contrary, the present value is lower than that reported by Mahmood et.al. (1991) in Pashmina goats treated with FSH or PMSG but higher than that reported by Pandey et.al. (1922) in Black Bengal goats treated with PMSG: PGF,a, hCG and by Doijode et.al. (1992) in Osmanabadi and crossbred goats treated with progesterone: PMSG. These variations on superovulatory response in goats in different studies might be due to differences in type and dose of exogenous hormone, nutritional status, breed, season and individual response.

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The mean number of ova/ embryos recovered per animal was 7.41 ± 1.75 which corroborates with the findings of Majumdar et.al. (1990) in Barbari goats and Pargaonkar et.al. (1992) in Osmanabadi and crossbred goats treated with PMSG. The present value was higher than that reported by Bonia (1992) in Assam local goats treated with PMSG. The mean rate of recovery of ova/embryos per animal was found to be 66.61 ± 5.30 per cent which is comparable with that obtained on day 3 post breeding by Agrawal et.al. (1982) Vadnere and Mani (1986) and Bonia (1992). The present value was higher than that obtained by Sarmah (1991) on day 5 post breeding in Assam local goats. The variation in recovery of embryos in different studies could be due to the variation in day of recovery, site of recovery and technique used. The number of ovulation per ovary may influence the percentage of recovery to a certain extent, since the fimbriated end of the oviduct may not be able to capture all the ova resulting in a reduction of recovery rate (Hafez, 1987).

## ACKNOWLEDGEMENT

The authors are grateful to the Dean, Faculty of Veterinary Science Assam Agricultural University, Khanapara, Guwahati for the facilities provided.

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## EFFECT OF 6 - METHOXYBENZOXAZOLINONE (6-MBOA) ON PMSG IN INDUCING SUPER OVULATION IN BLACK BENGAL GOATS\*.

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#### ABSTRACT

Adult, regularly cycling eight nonpregnant female black Bengal goats were pre-treated for ovarian hyper stimulation by oral progesterone for 10 days and divided into two groups. The treatment group (Group - A) consisting of 5 animals were given 6-Methoxy-benzoxazolinone (6-MBOA) @ 1 mg/kg of body weight along with 500 IU PMSG and 500 IU of HCG by intramuscular injections against the control group (Group-B) consisting of 3 animals which received only PMSG and HCG similar to that of Group-A without 6-MBOA. The mean number of corporalutea and Anovulatory follicles in Group-A were 11.30 + 1.16 and 1.7 + 0.21 and in Group-B they were  $4.5 \pm 0.99$  &  $1.5 \pm 0.22$ respectively. The corporalutea were significantly higher in Group-A where as the number of anovulatory follicles were evenly distributed in both the groups.

## The plant metabolite 6-

- Part of the thesis submitted to BCKV, Mohanpur, West Bengal for award of Ph.D. in Gynaecology and Obstetrics by the first author.
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- 2. Dept. of Animal Genetics & Breeding,

Methoxybenzoxazalinone (6-MBOA) was reported to stimulate reproduction in laboratory animals (Butterstain et.al., 1985; Nelson and Shiber, 1990). The effect of this metabolite in the farm animals was not extensively explored. In the present study, therefore, an attempt was made to understand the effect of this chemical (6-MBOA) on PMSG in inducing superovulation in black Bengal goats.

#### MATERIALS AND METHODS

The experimental animals comprised eight adult, regularly cycling, non-pregnant female black Bengal goats. They were maintained on oral progestogens (Medroxy progesterone acetate) for a period of 10 days. Then the animals were divided into two groups i.e. A and B. The treatment group (Group A) consisted of 5 animals which received by intramuscular injection 500 IU Pregnant Mare Serum Gonadotrophin (PMSG) and 1.0 mg per kg body weight of 6-MBOA dissolved in 0.5 ml. of ethanol and diluted to 3.0 ml with normal saline. The dose of 6-MBOA was calculated following Butterstein and Schyadler (1988) after some correction for body weight. The control

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group (Group B) consisted of 3 animals who received 500 IU PMSG and 0.5 ml of ethanol diluted to 3 ml with normal saline intramuscular injection.

All animals in both the groups received 500 IU Human Chorionic Gonadotrophin (HCG) intramuscularly 72 hrs. after PMSG treatment and after another 72 hrs. Iaparotomy was performed on mid ventral line to record the number of corporalutea (cl) and anovulatory follicle (af) in both the ovaries. The number of corporalutea and anovulatory follicles obtained in group A and Group B animals were compared applying students t-test (Snedecor & Cochran, 1966).

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## **RESULTS AND DISCUSSION**

The mean, S.E and the results of t-test of significance for corporalutea and anovulatory follicles obtained in the treated (Group A) and control (Group B) animals are described at the table.

The mean corporalutea in right ovaries of Group A and B animals were 11.60 + 2.11 and 5.0 + 1.53respectively but they did not differ significantly. The mean corporalutea in the left ovary was observed to be significantly (p < 0.05) higher in Group A (11.0 + 1.26) than in group B (4.0 + 1.53). But when the corporalutea of right and left ovaries were pooled, the mean difference between the group was found to be highly significant (P < 0.01) Group A animals recording higher mean value.

The mean of anovulatory follicles in right and left ovaries as well as when pooled revealed no significant difference between the treated and control groups. The over all mean follicles developed i.e. corpurluteum and anovulatony follicle across the right and left ovaries in Group A (6.5 + 1.24) was observed to be significantly higher then that in Group B (3.0 + 0.66).

The above results were similar to those reported by Schadler et. al., (1988) and Butterstein and Schadler (1988) in laboratory animals. The enhanced follicular development, ovulation and formation of CI in the 6-MBOA treated animals might be due to the synergestic effects of exogenous gonadotrophin administered with that of endogenous FSH secreted by hypophysis of the 6-MBOA treated animals.

It was also evident that the effect of chemical 6-MBOA was predominantly on the action of FSH rather than LH content of the PMSG as the number of anovulatory follicles in the treated and control groups did not differ significantly. The present study indicates a scope of use of the 6-MBOA as a drug modifier with FSH or PMSG in inducing superovulation in farm animals which in turn may be advantageously utilized in embryo transfer technology.

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nate il anovulatori	Group - A	Group - B	t-calculated	
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Rt. Ovary	11.60 ± 2.11 (5)	5.0 ± 1.53 (3)	2,18 N.S	
Lt. Ovary	11.0 ± 1.26 <sup>a</sup> (5)	4.0 ± 1.53 <sup>b</sup> (3)	3.46*	
Pooled/Ovary	11.30=1.16ª (10)	4.5 ± 0.99 <sup>b</sup> (6)	4.00**	
<b>Anovulatory Follicle</b>		the back to all bits	Sector I traile with	
RT. Ovary.	1.8 ± 0.37 (5)	1.33 ± 0.33 (3)	0.85 N.S.	
Lt. Ovary.	1.6 ± 0.24 (5)	1.67 ± 0.33 (3)	0.15 N.S	
Pooled/Ovary	1.7 ± 0.21 (10)	1.5 ± 0.22 (6)	0.612 N.S.	
Overall/Ovary	6.5 ± 1.24 <sup>a</sup> (20)	3.0 ± 0.66 <sup>b</sup> (12)	2.48*	

 Table: Mean + SE of corporalutea and anovulatory follicles in the

 6 - MBOA treated and control groups of goats

Figs in parenthesis denote no. of observations.

P < 0.05, P < 0.01, N.S.= Non significant.

The means in rows having different superscripts are significantly different. Group A = Treated, Group B = Control.

#### ACKNOWLEDGEMENT

The authors are highly indebted to M/s. Chem Biochem Research Inc. Utah, USA, for gifting the chemical 6-MBOA for the experiment and Dr. S.K. Haldar, Director A.R.D. Govt. of W.B. for permitting to carryout the work in Haringhata Farm.

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## ACETYLCHOLINE ESTERASE ACTIVITY AFFECTING PREGNANCY IN FARM ANIMALS

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Acetylcholine esterase (AchE) activity was estimated in whole blood of 176 cows of different breed and 44 huffaloes at the time of oestrus. In general pregnancy was highest (about 60.00%) when AchE value ranged between 123 to 135 mMol/ml/hr of whole blood in cows. whereas it was lower when AchE value was lower than about 100 mMol/ml/hr. No difference was observed due to breed characteristics. In contrast to cows. pregnancy in buffaloes was higher (63.16%) when AchE value was lower (35.50+1.72 mMol/ml/hr) whereas it decreased with increase in AchE value. This experiment shows that breed does not influence AchE value significantly but species certainly have effect on AchE activity and accordingly on pregnancy.

Blood level of acetylcholine esterase (AchE) may have some relationship with ovulation (Edward, 1974). Therefore present study was undertaken to assess possible changes in blood plasma AchE activity in cows and buffaloes during oestrus and its relation with pregnancy.

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#### MATERIALS AND METHODS

Study was carried out on 30

Holstein Friesian, 62 halfbred (F x H), 84 three breed cross ( $\frac{1}{2}$  F x  $\frac{1}{4}$  J x  $\frac{1}{4}$ H;  $\frac{1}{2}$  F x  $\frac{1}{4}$  B x  $\frac{1}{4}$  H) cows and 44 buffaloes. Oestrus was detected by vasectomised bull. Animals observed in standing oestrus with clear and transparent discharge were included in experiment. Animals were inseminated after 10-12 hr. of oestrus symptoms and pregnancy was confirmed after 45-60 days post Al.

Two ml. whole blood was collected in haparinised tube from each animals at the time of oestrus. Sample was brought to the laboratory under ice and AchE activity was estimated according to method described by Bowman and Rand (1980) and expressed as the amount of acetylcholine (mMol/ml/hr) hydrolysed by AchE enzyme.

## **RESULTS AND DISCUSSION**

the results are summarised in Table. In general pregnancy rate decreased in cows having low level of AchE at oestrus. Among Holstein Friesian, half bred and three breed crosses pregnancy rate was 60.00, 52.94 and 61.51% with AchE level 135.75 +1.70, 123.58 +2.04 and

121.75 + 1.62 mMol/ml/hr respectively. This shows that there is no effect of breed on AchE activity. Ramachandra et al. (1992) also reported AchE activity 112  $\pm$ 13 enzymes unit/ml in crossbred after 6 hr. of onset of oestrus but did not study relationship with pregnancy. From our study it is clear that ovulation in farm cows mostly occurs when AchE value is around 120 mMol/ml/hr. In contrast to cows, in buffaloes, AchE value was

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always very low both during high pregnancy ( $35.50 \pm 1.72$ ) and low pregnancy ( $60.66 \pm 3.38 \text{ mMol/ml/hr}$ ) as compared to cows (Table). No such work has been mentioned in literature but species difference may be one of the factor, which warrants further investigation. However, the peculiar observation lend support to the view that a particular level of AchE enzyme is necessary for ovulation in farm animals.

Table : AchE	activity	(mMOI/ml./hr.)	and cor	nception in	farm animals
	T	Lat.		0	Alexander F

Breed / Species	Total number of animals	Number of pregnant animals	Conception Rate (%)	Mean AchE activity ±SE
Holstein friesian	15 15	4 9	26.66 60.00	110.00 ± 5.51 135.75 ± 1.70
Halfbred (F x H)	29	9	31.03	87.66 ± 3.30
and Achill activity, was	34	18	52.94	123.58 ± 2.04
Three breed cross	27	7	25.92	88.28 ± 3.87
	38	24	61.51	121.75 ± 1.62
andmin (ministration)	19	6	31.58	158.96 ± 12.38
Buffaloes	- 19	12	63.16	35.50 ± 1.72
- MONESUSED ONA -	25	6	24.00	60.66 ± 3.38

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## EFFECT OF PROGESTERONE SUPPLEMENTATION ON CONCEPTION RATE IN REPEAT BREEDER CATTLE

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#### ABSTRACT

The effect of progesterone on conception rate was studied in 48 repeat breeder crossbred cows. 250 mg. progesterone was administered at 5th 12th and 19th, day after A.I. in treatment group. Animals both in treated and control groups were free from detectable genital infection and other reproductive abnormalities. First service conception rate was 39.13% in treated animals (n=23) while it was only 8.00% in controls (n=25). Thus administration of progesterone in repeat breeder crossbred cattle at first breeding increases the conception rate of treated group by 31.13% over the control for first service conceptions. The overall conception rate of 65.21% for treated group was comparatively higher than overall conception rate of 48% for control group.

Repeat breeding is the major fertility problem in a dairy farm. As much as 10% of heifers are culled every year due to repeat breeding (Johnson et at., 1958). In the absence of infectious diseases of reproductive system, embryonic mortality is the most important factors for repeat breeding. Approximately 40% of bovine embryos are lost during days 8-18 after A.I. (Diskin and Sreenan, 1980; Sreenan and Diskin, 1983). The maintenance of progesterone secretion by viable corpus luteum is vital to early pregnancy. Cattle which conceive after A.I. have been shown to have a more pronounced rise in progesterone levels in plasma or milk during the early letual phase than cattle which do not conceive (Henricks et al., 1971).

The objective of this study was to determine the effects of progresterone on conception rate in repeat breeder crossbred cattle.

#### MATERIALS AND METHODS

Forty eight repeat breeding crossbred animals were selected from the dairy farm at Indian Veterinary Research Institute, Izatnagar. All the animals were examined per rectally and only those animals which were free from abnormalities, detectable infection and diseases were taken for the experiment.

The animals were bred twice during the estrous period by A.I. After the animals were bred, alternate animals were treated with a progesterone (Duraprogen, Unichem

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Lab. Ltd., Mumbai) The treated group received 250 mg. of progesterone intramuscularly on the 5th, 12th and 19th days after A.I. All the animals were examined for pregnancy by rectal palpation at 45 days and 150 days after breeding.

## **RESULTS AND DISCUSSION**

Out of 48 repeat breeding crossbred cattle, twenty three animals treated with 250 mg. progesterone 9 conceived to first service with a conception rate of 39.13%. Three animals conceived to second service (21.42%) and two to a third (27.27%) and one to a fourth service without further treatment. The 23 animals

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conceived on a total of 2.2 services with a conception rate of 65.21% (Table 1).

In control, 25 animals conceived on a total of 4.08 services with a conception rate of 48%. The other animals in both groups returned to estrous on the 21st days were rebred till they conceived.

The difference in first service CR and overall CR between treated and control group were highly significant (p<0.1) and significant (P< 0.05) respectively by Chi-Square test.

The CR for average breeding efficiency as reported by Andrews (1949) was 55% or 1.8 services per

Table 1. Influence of	progesterone	supplementation	n on CR in
repeat	breeder cros	sbred cattle.	

OPOUR	Graelains		t different	Overall	Service /		
GROUP	Animal No.	I	II	III	IV	CR	conception
Treated	23	39.13**	21.42	27.27	11.11	65.21*	2.2
Control	25	8.00**	4.34	18.18	27.77	48.00*	4.08

\*(P<0.05); \*\*(P<0.1)

conception which is closer to the result obtained in treated group. The number of services per conception reported by other worker in normal breeding animals were 1.7 to 2.5 (Johnson et al., 1958) which is in agreement with the results of treated group. The improvement in overall CR (17.21%) by supplementation of progesterone is in agreement with the report of

Sreenam and Diskin (1983). However it is in contrast to the report of Vancleef et al, (1989).

Several workers reported that embryonic mortality on day 8 and on day 18 as the major cause of repeat breeding (Sreenam and Diskin, 1983 and Peters, 1996). It may be due to lower levels of progesterone during

this period in repeat breeding cattle (Dutta et al., 1989) and the supplementation of progesterone at this stage may be beneficial (Robinson et al., 1989. Sreenam et al., (1980) and Diskin and Sreenam (1986) found increased embryo survival in both normal and repeat breeding cows with progesterone supplementation.

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# REPRODUCTIVE PERFORMANCE OF HOLSTEIN HALFBRED AND JERSEY HALFBRED COWS IN THE PLAINS OF EASTERN INDIA

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## ABSTRACT

The data on the reproductive traits of 44 Jersey Halfbreds and 23 Holstein Halfbreds in their first parity, born during the period 1977-96 at the Institute Farm were analysed by least squares technique for the effect of genetic and nongenetic factors on reproduction traits viz. age at first calving, weight at first calving, service period, inter calving period and number of inseminations required per conception. The difference between the two genotypes was significant (P<0.05) with respect to age at first calving only. Jersey Halfbreds had lower age at first calving (1063.6+19.1 days) than the Holstein Halfbreds (1160.5+27.5) days). Period of calving significantly affected age at first calving, while season of calving had no significant effect on any of the traits. Service period had significant and positive correlation with Inter-calving period (0.85) and number of inseminations per conception (0.30), while inter-calving period had a negative correlation (0.24) with the latter. The study implies that there is scope of further reduction in the service period and inter-calving period through better artificial insemination management.

In India, Holstein and Jersey

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breeds of sires were used extensively for crossbreeding with the indigenous breeds and non-descripts for improving milk production. The performance of the crossbreds were variable in different agro-climatic zones. Reproductive traits are considered to be important because of their positive association with breeding efficiency and milk production (Arora et al., 1996,; Barwe et at., 1996.; Jadhav and Khan, 1996). Hence, an attempt has been made in this paper to make a comparative evaluation of the reproductive performance of Holstein halfbred and Jersey halfbred cows for their suitability to reproduce and yield under hot-humid agroclimatic condition of West Bengal.

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# MATERIALS AND METHODS

The data for the present investigation were based on the reproductive records of 23 Holstein halfbreds and 44 Jersy halfbreds in their first parity, which were born at the Institute Farm during the period 1977 to 1996. The breed of the Sire was Holstein or Jersey, while the breed of the dam was Red Sindhi/

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Tharparkar. The reproductive traits included in the study were age at first calving (AFC), weight at first calving (WFC), service period, intercalving period (ICP) and number of inseminations required per conception. The years were grouped into two periods of 10 years each with three seasons of calving viz. winter (November - February), summer (March - June) and monsoon (July -October). The effect of genetic and non-genetic factors e.g. genotype, period of calving and season of calving were analysed by least squares analysis of variance technique. The group means were tested for significance. The phenotypic correlation between the traits were estimated and tested for significance.

# **RESULTS AND DISCUSSION**

The least squares Mean and Standard Error (S.E) for the traits and the result of the analysis of variance of the traits are given in Table. There was significant difference between the two genotypes with respect to age at first calving. The effect of period of calving was significant on age at first calving only, while season of calving had no significant effect on any of the traits. The leastsquares Mean of age at first calving in Jersey halfbred and Holstein halfbred cows were

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1063.6+19.1 and 1160.5+27.5 days respectively. The present estimates are lower than the estimates reported for Jersey x Hariana and Holstein x Hariana F2 crosses in West Bengal (Bala, 1983). Jersey Halfbreds were superior to Holstein Halfbreds with respect to age at first calving, in contrast to the results to Bala (1983) and Yazdani et al. (1993), who had reported that there was no significant difference in age at first calving between the Holstein halfbreds and Jersey halfbreds in their studies.

Service period had significant and positive correlation with Intercalving period (0.85) and number of inseminations per conception (0.30), while inter calving period had a negative correlation (-0.24) with the latter. The results indicated that there was scope of further reduction in intercalving period through better management of artificial insemination service.

The result tends to conclude that Jersey halfbreds scored an advantage over Holstein halfbreds with respect to age at first calving, while both the genotypes have shown to have acquired equal adaptability to the hot-humid climatic condition of Eastern India with respect to other reproductive traits.

Effect	A.F.C. (days)	W.F.C. (Kg.)	Service period (days)	I.C.P. (days)	No. of Al / concep
/u	1112.0±17.6ª	333.8±12.3	182.7±17.5	444.0±14.1	1.6±0.16
	(67)	(51)	(49)	(48)	(48)
J.H abon	1063.6±19.1 <sup>b</sup>	317.8±9.2	182.1±21.3	438.8±17.3	1.6±0.19
	(44)	(40)	(29)	(28)	(28)
н.н	1160.5±27.5×	349.9±20.6	183.3±26.1	449.2±20.8	1.6±0.19
	(23)	(11)	(20)	(20)	(20)
Period 1	1184.3±24.3 <sup>y</sup>	326.2±17.9	205.2±23.4	451.9±19.2	1.7±0.21
	(29)	(14)	(25)	(24)	(24)
Period 2	1039.8±29.7	341.4±16.6	160.2±30.5	436.1±24.4	1.5±0.27
	(38)	(37)	(24)	(24)	(24)
Winter	1100.6±29.4	319.9±17.2	228.0±29.2	472.9±23.4	1.4±0.26
	(32)	(26)	(21)	(20)	(20)
Summer	1121.6±37.6	360.8±22.8	127.1±37.8	409.2±30.3	1.9±0.34
	(21)	(15)	(17)	(17)	(17)
Monsoon	1113.9±34.1	320.8±18.8	193.0±35.3	449.9±28.5	1.5±0.32
	(14)	(10)	(11)	(11)	(11)

Table - 1 : Leastsquares Mean ±SE of Reproductive Traits

Note: The figures are presented as Mean + S.E (No. of Observation). Group Means with similar superscripts do not differ at P<0.05.

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# MACRO MINERAL LEVELS IN CYCLIC, POST-PARTUM ANESTROUS AND REPEAT BREEDING LOCAL COWS IN LOWER BRAHMAPUTRA VALLEY OF ASSAM

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#### ABSTRACT

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An attempt was made to study the macromineral status in cyclic, postpartum an anestrous and repeat breeding local breeds of cows in lower Brahmaputra Valley of Assam. The concentrations of marcrominerals were highest in Nalbari subdivision and lowest in Dhubri subdivision. When comparison was made among different groups, the levels of macromineral were found to be significantly (p < 0.01) highest in cyclic cows compared to the repeat breeding and post-partum anestrous cows.

Minerals play an important role in the regulation of reproduction and production of domestic animals. Minerals like calcium, phosphorus and magnesium also influence the ability of animal to utilize other microminerals. The influence of these minerals on certain enzyme system may affect reproductive efficiency (Dhoble and Gupta, 1986), which might be reflected in lower blood levels of them. The present investigation

1. Assistant Professor, Dept. of Physiology and biochemistry.

 I/C Associate Dean, Lakhimpur College of Veterinary Science, AAU, North Lakhimpur. was, therefore, designed to investigate the calcium, inorganic phosphorus and magnesium in cyclic, post-partum anestrous and repeat breeding cows of local breed in Lower Brahmaputra Valley of Assam.

#### MATERIALS AND METHODS

Lower Brahmaputra Valley, one of the six agro-climatic zone of Assam, representing 25.75 per cent of total state area and possessing highest livestock population of about 29.04 per cent (Livestock Census of Assam, 1988) have been selected for collection of blood samples from local breed of cows for estimation of some of the macro minerals viz. calcium. inorganic phosphorus and magnesium. The zone is comprised of twelve sub-divisions, of which sixdivisions namely Rangia, Nalbari, Barpeta, Goalpara, Bongaigaon and Dhubri were selected for collection of samples. From each sub-divisions two cluster of villages were selected randomly. Each cluster of villages consist of five numbers of adjacent villages. Thirty cows from each cluster of villages were selected and they were divided into three, categories of

ten each in cyclic. (conceived to 1 to 2 services), post-partum anestrus (even after 60 day post partum and with inactive ovaries), and repeat breeding (failed to conceive even after three services and with no genital abnormality) according to the reproductive status. The animals were fed some concentrate (commercially available) mixture, paddy straw and allowed to graze in the grazing fields, mostly dominated by carpet grass (Axonopus affinis) and Doob grass (Cynadon dectylon). A total of 360 blood samples were collected from the three categories of animals. Serum was separated from the blood samples and processed (Fick et al., 1979) and analysed for macro mineral contents Flams Atomic Absorption bv Spectrophotometry. The data were analysed statistically (Snedecor and Cochran, 1973).

## **RESULTS AND DISCUSSION**

The circulatory levels of serum calcium, inorganic phosphorus and magnesium in cyclic, post-partum anestrus and repeat breeding cows are presented in Table

The concentration of different macrominerals differed significantly (P< 0.01). among different subdivisions, with the highest values observed in Nalbari sub-division and lowest values in Dhubri sub-division.

The overall average values for serum calcium, inorganic phosphorus and magnesium were

maximum in normal cyclic cows and minimum in postpartum anestrous cows and this difference was statistically significant (P < 0.01) However, serum calcium, inorganic phosphorus and magnesium levels between postpartum anestrous and repeat breeding cows did no vary significantly. The higher level of serum calcium in cyclic cows in the present study might be due to fluctuating levels of oestrogen in the cyclic animal. The lower level of serum calcium in postpartum anestrous and repeat breeding cows might be due to the failure of endocrine system to mobilize the body calcium which leads to reproductive failure. Balakrishnan and Balagopal (1994) also suggested that mean serum calcium level was significantly low in repeat breeders.

The lower level of serum inorganic phosphorus in post-partum anestrous and repeat breeding cows in the present study is in close conformity with the findings of Naidu and Rao (1982) and Quayam et al. (1988) who reported that marginal deficiency of phosphorus in blood serum was sufficient to cause disturbance in pituitary-ovarian axis without manifestation of specific deficiency symptoms. Shrivastava and Kadu (1995) observed lower levels of inorganic phosphorus in serum of anestrous cows than cyclic cows. Fable : Serum Macromineral Levels in Cyclic, Post-partum Anoestrus and Repeat Breeding of Different Sub-divisions

The higher level of serum magnesium in cyclic cows in the present study is in close agreement

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Table : Serum Macromineral Levels in Cyclic, Post-partum Anoestrus and Repeat Breeding of Different Sub-divisions

Macro-minerals (mg%)	Category of Animal	Rangia	Nalbari	Barpeta	Goalpara	Bongaigaon	Dhubri	Overall
Calcium	Cyclic	10.77 <sup>+</sup> ± 0.24	11.01° ± 0.23	10.92 <sup>th</sup> ± 0.17	10.62 <sup>d</sup> ± 0.19	10.71" ± 0.21	10.97 <sup>4</sup> ± 0.14	10.73° ± 0.08
	Postpartum anoestrus	9.79" ± 0.32	10.16 <sup>b</sup> ± 0.20	9.25° ± 0.51	9.58° ± 0.21	9.21° ± 0.66	8.90 <sup>4</sup> ± 0.30	9.54 <sup>1</sup> ± 0.22
inter a	Repeat breeding	10.44° ± 0.34	10.52° ± 0.34	9.92 <sup>6</sup> ± 0.33	9.88 <sup>ki</sup> ±1.11	9.62 <sup>dl</sup> ± 0.38	9.43 <sup>4</sup> ± 0.52	9.95°±0.18
	Cyclic	4.58" ± 0.14	4.61°± 0.13	4.12 <sup>b</sup> ± 0.16	4.07 <sup>b</sup> ± 0.17	3.88 <sup>k</sup> ± 0.18	3.80° ± 0.19	4.22° ± 0.07
Inorganic phosphorus	Postpartum annoestrus	3.45° ± 0.24	4.05 <sup>b</sup> ± 0.18	3.47° ± 0.29	3.79" ± 0.31	3.21° ± 0.19	2.94° ± 0.36	3.48' ± 0.12
1 American	Repeat breeding	4.01°±0.55	4.09° ± 0.29	3.44k ± 0.31	3.53 <sup>6</sup> ± 0.22	3.26 <sup>k</sup> ± 0.29	3.25° ± 0.23	3.62 <sup>4</sup> ± 0.13
	Cyclic	3.43°± 0.17	3.46"±0.17	3.14 <sup>4</sup> ± 0.21	3.13 <sup>t</sup> ± 0.12	3.12 <sup>6</sup> ± 0.15	3.12 <sup>b</sup> ± 0.20	3.19° ± 0.07
Magnasium	Postpartum anoestrus	2.46" ± 0.42	3.42° ± 0.45	2.44° ± 0.43	2.25" ± 0.28	2.01° ± 0.22	2.00° ± 0.16	2.34 <sup>1</sup> ± 0.14
SAUT	Repeat breeding	3.12° ± 0.30	3.42 <sup>4</sup> ± 0.37	2.42" ± 0.30	2.35° ± 0.30	2.35° ± 0.18	2.19" ± 0.18	2.56 <sup>1</sup> ± 0.12

Means within a row with different superscripts (a, b, c, d) differ significantly (P < 0.01).

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Overall group means within a column with different superscripts (e, f, g) differ significantly (P < 0.01).

with the findings of Dindorkar and Kohli (1979). Venkatamuni Chetty and Ramamohan Rao (1986) also reported significantly higher serum magnesium level in cyclic cows than that of anestrous cows. Further it was stated that magnesium plays a key role in the imbalance of other mineral status of repeat breeding animals (Balakrishnan and Balagopal, 1994). Indian J. Anim. Reprod., June, 2001

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The concentrations of serum calcium and magnesium were within the normal range but the value observed for inorganic phosphorus in anestrous and repeat breeding cows were found to be deficient against the critical value of 4.0 mg% (Mc Dowell et al., 1984).

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CLINICAL OBSERVATIONS AND SUCCESS OF TREATMENT OF UTERINE TORSION IN BUFFALOES\*

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#### ABSTRACT

The present study retrospectively evaluates the clinical findings and survival rate following treatment in 47 uterine torsion affected buffaloes. Uterine torsion was most frequently encountered in pluriparous buffaloes with complete gestation periods. Right side post-cervical uterine torsion was seen in more than 90% affected buffaloes. Detorsion of uterus was achieved in 84% animals subjected to rolling. It was more successful in animals with complete gestation (86.6%) than with incomplete gestation (75%); the per-vaginal deliveries in the respective groups being 92.3 and 66.6%. The survival rate was maximum (80.8%) in buffaloes where cervix dilated and fetus was delivered pervaginally following detorsion. Lower survival was seen in buffaloes subjected to Caesarean either due to failure of detorsion or failure of cervical dilation.

Torsion of uterus, if not intervened at an appropriate time may lead to death of fetus, dam or of both. This warrants thorough knowledge and

Part of M.V.Sc. thesis submitted to P.A.U., Ludhiana evaluation of the clinical symptoms for prompt treatment. Owing to delay in the treatment, utero-omental adhesions may develop that render the animal fit for only surgery. Although, rolling of the dam has been found to be successful for uterine detorsion, yet, treatment success may vary depending upon the clinical evaluation.

The present study evaluates retrospectively the clinical findings and success of treatment in buffaloes suffering from torsion of uterus.

#### MATERIALS AND METHODS

Forty seven buffaloes affected with uterine torsion presented for treatment at PAU Veterinary Clinics were utilised. At presentation following anamnesis in respect of age, calving status, stage of gestation and onset of labour pains, per-vaginal and perrectal examination were done to diagnose the exact condition. Thirty eight buffaloes without utero-omental adhesions were subjected to detorsion of uterus through Sharma's modified Schaffer's method (Singh and Nanda, 1996). Following detorsion the dilation of cervix was assessed and the fetal delivery was achieved. Nine other

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buffaloes were subjected to Caesarean section performed at the conventional site. Survivability of the dam following treatment was considered as the criteria for success of the procedure.

# **RESULTS AND DISCUSSION**

Clinical Findings : High incidence of uterine torsion was recorded in pluriparous (68.06%) as compared to the primiparous buffaloes (31.9%) in this study. Among total cases, 74.47 per cent had completed their gestation while the gestation period was incomplete in 25.53 per cent Clinical examination buffaloes. revealed the preponderance of right side uterine torsion (93.62%) with 95.74 per cent buffaloes having postcervical uterine torsion. 180 (46.8%) followed by 90 (21,28%) and 360 (19.15%) were the most common degrees of rotation. No variation in the sex of the calf was observed. Male to female calf ratio was 5.5 to 4.5. Majority of the calves (87.23%) were dead at delivery.

has earlier been observed (Vasishta, 1983). Findings of complete gestation period in majority of buffaloes corroborated the earlier reports of Arthur et.al (1982) and Prabhakar et.al (1994) indicating that the torsion occurred more frequently around parturition. The occurrence of right

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side post-cervical uterine torsion in more than 90 per cent buffaloes in the present study was contrary to the findings of Foud and El-Sawaf (1964). The morphology and attachment of broad ligaments played an important role in causing uterine torsion (Singh et al 1992) since such buffaloes were found to have broad ligament with poor muscular development.

Success of Treatment : Detorsion of uterus was tried in 38 buffaloes (80.15%) while Caesarean section had to be performed in 19.15 per cent animals owing to the presence of utero-omental adhesions. The detorsion of uterus was achieved in 32 buffaloes (84.21%)

The cervix was found to be fully dilated in 56.25 per cent buffaloes at detorsion whereas in 31.25 per cent animals, the incompletely dilated cervix dilated within 10 hours after detorsion of uterus following treatment with calcium borogluconate (450 ml) and/or oxytocin (50 I.U.). The fetal delivery was achieved in them pervaginum. Remaining buffaloes whose cervix failed to dilate were delivered through Caesarean section.

The success of detorsion in the present investigations was higher in cases with complete (86.60%) than in cases with incomplete gestation periods (75.0%). Likewise, higher pervaginal deliveries were achieved (92.3%) in buffaloes with completed

gestation whereas the same was achieved only in 66.6 per cent buffaloes having incomplete gestation periods. Two buffaloes each whose cervix did not dilate following detorsion with complete or incomplete \* gestations were delivered through Caesarean section.

The overall survival rate in the present investigation was found to be 72.34 per cent. The animals having fully dilated cervix at detorsion had higher survival (88.8%) than the animals where cervicx dilated subsequently and per-vaginal delivery was achieved (70.0%) or it did not dilate and Caesarean section was performed (50.0%). In buffaloes where direct Caesarean section was performed without attempted detorsion, the survival rate was 66.6 per cent. 36

The success of detorsion of uterus in the present study was in agreement to the findings of Dhaliwal et al (1991) and was higher than that reported by Mannari and Tadkod (1976) and Pattabiraman et al (1979). Selective rolling following careful diagnosis might have resulted into higher success rate. Delay in the treatment often leads to linear decline in success of detorsion of uterus (Prabhakar et al 1995). Development of utero-omental adhesions, often beyond 96 hours of the onset of problem, rendered the animal fit for surgery only.

Finding of fully dilated cervix at detorsion in more than 50 per cent cases substantiate the findings of others about the occurrence of the condition near the term or beginning of second stage of labour (Arthur et al 1982). Failure of cervical dilation in some of the animals might be due to delayed presentation for treatment or higher degree of torsion leading to tissue degeneration (Singla, 1988). Prabhakar et al (1997) have observed poor dilation of cervix in pre-cervical uterine torsions even within 36 hours of presentation.

Higher survival rate following detorsions and per-vaginal delivery with fully dilated cervix than those with incompletely dilated cervix confirmed the earlier observations (Nanda et al 1985). Lower survival in cases subjected to Caesarean section due to non-dilation of cervix reflected cumulative stress of dystocia, rolling and surgery. Rolling for detorsion (Ghuman et al 1998) and Caesarean section (Prabhakar, 1995) have been found to be highly stressful as reflected by plasma cortisol levels. As observed, avoiding attempted detorsion before surgery in delayed cases can improve dam survivability. The overall survival rate indicates that provided the cases of uterine torsion are treated early and properly assessed before any treatment, detorsion and per-vaginal delivery can be achieved, while the delayed cases often require surgery for fetal delivery.

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# TREATMENT OF RETENTION OF PLACENTA IN RELATION TO SUBSEQUENT REPRODUCTIVE AND PRODUCTIVE PERFORMANCE IN FRIESWAL COWS

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## ABSTRACT

All the cows expelled their placenta significantly earlier (p < 0.05) in the Vitacept + Nutrimilk treated group (5.82 + 0.26 hours) as compared to untreated (8.66 0.56 control hours). + Disappearance of lochia / conception rate were better in the treated group. Involution of uterus / occurrence of first postpartum estrus were significantly earlier in the treated group. In treated group service period (72.60 + 3.13 days) was significantly less as compared to untreated control group (87.30 + 4.30 days). There was an improvement in 90 days milk production in treated (1344.4 + 68.17 litres) than the untreated control group (1012.4 + 85.17 litres).

The mean serum calcium / inorganic phosphorus did not vary significantly during placental retention (9.92 + 0.10 mg % / 5.08 + 0.06 mg %) and normal calvings (10.46 + 0.29 mg % / 5.28 + 0.16 mg %). During retention of placenta the level of haemoglobin and differential leucocyte count did not show any significant difference compared to normal calvings. In retained cotyledons, the histomorphology revealed various stages of necrosis in trophoblastic cells. In some sections, an increased number of

Part of MV Sc. Thesis of the first author submitted to the Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur. binucleate cells were also preser

It has been reported that during retention of placenta changes occur in the calcium - inorganic phosphorus ratio, haemogram and histoarchitecture of retained cotyledone (Roberts, 1986; and Wervan et al., 1992). Hence, the present investigation was undertaken to study all these factors during placental retention along with the treatment response in relation to subsequent reproductive and productive performance in Frieswal cows.

#### MATERIALSAND METHODS

The study was conducted on the 52 Frieswal (Holstein Friesian X Sashiwal) cows belonging to the Military Dairy Farm, Jabalpur. Twelve Frieswal cows with equal number of animals in the group with retention of placenta and normal calvers (control) were employed to study the biochemical, haematological and histological parameters. About 10 ml blood was collected by veinpuncture in a 20 ml test tube within 12-24 hours postpartum. An aliguote of the same

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was used for preparing the smear for differential leuocyte count and estimating haemoglobin percent using the Sahli's Haemoglobinometer (Jain, 1986). Rest of the blood was utilised for serum separation for the estimation of calcium and inorganic phosphorus (Oser, 1976).

For the histological studies the placental cotyledons were collected from the cows expelling their fetal membranes within 8 hours postpartum and those in which the membranes were removed manually 48 hours after calving. The collected tissues were processed as per standard procedure. The sections were stained using haematoxylingeosin stain (Luna, 1968) and the slides were examined under 100 x and 400 x magnifications.

Totally, 40 pregnant Frieswal cows of the farm were allotted randomly with equal number to the 2 groups. To the treated group of cows Vitacept (Pfizer India Ltd.) was administered at the does rate of 5 ml, weekly, I/M plus Nutrimilk (Concept Pharma Ltd.) pulv, 50 g o.d., orally, after 8 months of pregnancy till calving. The second group was maintained as untreated control.

The time taken for expulsion of placenta was recorded to know the efficacy of drug with respect to the duration of expulsion, whether it was within 6-12 hours, 12 to 24 hours or more than 24 hours postpartum. Attempt to remove the placenta

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manually were undertaken only after 48 hours of the calving as per the procedure outlined by Arthur et al, (1996) followed by intrauterine administration of antibiotics till complete recovery in both the group. The external genitalia were routinely examined for vaginal lochial discharge for its nature and day it ceased. The animals with abnormal discharge were treated with intrauterine administration of Lugol's lodine till complete recovery. The involution of uterus was studied in cows by regular gynaeco-clinical examination on 2,3,7,9 and 15 day of calvings and daily thereafter till the day of complete uterine involution as per the criterion adopted by Morrow et al. (1969).

The interval from calving to appearance of first postpartum estrus was recorded in all the cows. The animals in heat were inseminated interval from calving to conception was recorded in both the group of animals along with the services per conception. The pregnancy was diagnosed gynaeco-clinically after two month of insemination. In both the groups 90 days milk production was recorded. The haematological and biochemical findings, and the efficacy of Nutrimilk and Vitacept treatment were compared with untreated control in relation to various reproductive traits using Students 't' test (Snedecor and Cochran, 1994).

# **RESULTS AND DISCUSSION**

Table 1 presents biochemical

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and haematological observations during retention of placenta in Frieswal cows. The mean serum calcium / inorganic phosphorus did not vary significantly during placental retention and normal calvings Roberts (1986) stated that the calcium and inorganic phosphorus deficiency causes uterine intertia and atony which results in a high incidence of the disease. However, Choudhary et al. (1993) did not get any appreciable change in the inorganic phosphorus contents in the cows with retention of placenta.

During retention of placenta the levels of haemoglobin and differential leucocyte count did not show any significant difference compared to normal calvings, Jain (1986) described that the lymphocyte and monocytes level get elevated with 'shift to the left' in cows with infection. However, as found in the present study, Benysek and Kudlac (1971) also did not record any significant difference in the haemogram during the retention of placenta.

In the present study the histomorphology of retained cotyledons revealed various stages of necrosis in trophoblastic cells. These findings are in accordance with the Bjorkman and Sollen (1960). In some section increased number of binucleate cells were observed in retained placentome. These observations agree with the findings of several workers (Williams et al., 1987; and Al-Sadi et al., 1994). It was concluded that the presence of

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binucleate cells in the placentome during peripartal period was an essential prerequisite for normal separation of after births.

As presented in Table 2, all the cows expelled their placenta significantly earlier (p < 0.05) in the Vitacept + Nutrimilk treated group (5.82 + 0.26 hours) as compared to untreated control (8.66 + 0.56 hours). Disappearance of lochia, involution of uterus, occurrence of first postpartum estrus and number of services per conception were significantly less in treated group than untreated control. The beneficial effect of the treatment was probably due to vitamin A, D,, E and Selenium ingredients present in the drugs. Valyushkin and Kurzeka (1991) reported that administration of retinol through the intramuscular route decreased retention of placenta incidence by 16.6%, fertility was increased by 13.4% and service period was 13 days shorter. Similarly, Erskind et al. (1997) also observed that administration of vitamin E reduced the incidence or retention of placenta.

There was improvement in 90 days milk production in treated (1344.4 + 68.17 litres) than the untreated control group (1012.4 + 85.17 litres). These findings are in accordance with the observation of Pavlicek et al. (1979), who reported rise in the milk yield from 755 kg to a record of 6296 kg per cow after administration of selemium and vitamins to prevent placental retention in high yielding dairy cows.

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 Table 1: Biochemical and haematological observations during retention of placenta in Frieswal cows (Mean + SE)

Parameters	Retention of placenta $N = 6$	Normal calvings N = 6
Calcium (mg%)	9.92 ± 0.10	10.46 ± 0.29
Ignorganic Phosphorus (mg%)	5.08 ± 0.06	5.28 ± 0.16
Calcium: inorganic phosphorus	1.95:1.00	1.97:1.00
Haemoglobin (g%)	9.93 ± 0.07	10.06 ± 0.10
Differential Leucocyte count	a realization	
Neutrophil (%)	36.87 ± 1.17	38.95 ± 0.75
Lymphocyte (%)	54.38 ± 0.18	52.24 ± 0.80
Monocyte (%)	3.13 ± 0.45	2.89 ± 0.34
Eosinophil (%)	4.23 ± 0.45	4.89 ± 0.31
Basophil (%)	0.50 ± 0.17	0.40 ± 0.10

# Table 2: Response of Vitacept and Nutrimilk treatment in retention of placenta in Frieswal cows (Means + SE)

Observations	Vitacept + Nutrimilk N = 20	Untreated control $N = 20$		
Animals expelling placenta within 8 hours postpartum(%)	100	100 m 11 at at 80		
Duration of expulsion of placenta (Hours)	5.82 + 0.26*	8.66 + 0.56		
Disappearance of lochia (Days)	8.91 + 0.76	9.90 + 1.25		
Uterine involution (Days)	23.17 + 0.37*	29.35 + 0.35		
First postpartum estrus (Days)	56.17 + 4.38*	60.90 + 5.76		
Number of services per conception	1.80 + 0.28	2.40 + 0.17		
Service period (Days)	72.60 + 3.13**	87.30 + 4.30		
90 days milk production (Litres)	1344.40 + 68.17**	1012.4 + 85.17		

\* P < 0.05 \*\* P < 0.01

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# MICROBIAL FLORA AND ANTIBIOGRAM PATTERN OF THE UTERINE ISOLATES FROM CROSSBRED COWS WITH ENDOMETRITIS

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## ABSTRACT

The type of microorganisms involved in endometritis and their antibiotic sensitivity pattern was studied in 205 crossbred cows. Staphylococcus species were most prevalent followed by Streptococcus among gram positive organisms. Among gram negative organisms E. coli and pseudomonas were prevalent. The study revealed that highest number of isolates were sensitive to Gentamicin (80.98%) while only 20% isolates were sensitive to Oxytetracycline.

Endometritis occurs most commonly during the postpartum period. The husbandry and sanitation practices commonly employed in the management of dairy cows at parturition are inadequate and expose the uterus to a broad range of bacterial

Part of M.V.Sc., thesis of first author submitted to Acharys N.G. Ranga Agricultural University, Hyderabad.

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contamination leading to increase in incidence of postpartum endometritis in crossbred cows. An investigation was undertaken to study the microbial flora and their antibiogram pattern.

## MATERIALS AND METHODS

Studies were conducted on crossbred cows presented at six Veterinary dispensaries in and around Chittoor town of Andhra Pradesh. Two hundred and five crossbred cows affected with endometritis were selected for the present study. Endometritis was diagnosed based on history and gynaecological examination.

Uterine fluids were collected by aspiration under sterile conditions using normal saline. Primary and sub culturing was done employing routine methods. Most predominantly occurring isolates from each sample were identified as per standard procedures based on morphology, staining reaction, and by a battery of biochemical tests (Cowan, 1974).

Antibiotic sensitivity test was conducted using various antibiotic

Biodiscs (Hi-media) and Muller -Hinton agar (Hi-media) plates.

# **RESULTS AND DISCUSSION**

Regarding types of bacterial isolates identified in the uterine fluids Gram positive organisms were isolated from 114 (55.16%) cows and gram negative organisms from 68 (33.17%) cow while 23(11.22%) cows were found to have mixed infection. The present investigation is in close agreement with the report of Venkateswarlu et.al (1983).

Among the gram positive organisms Staphylococcus (30.24%) was found to be the most predominant causative organism of endometritis followed by Streptococus (20%). The predominance of Staphylococcus was also reported by Jacob (1993), on the other hand a very low percentage of incidence (3.89%) was reported by Ambrose and Pattabiraman (1993). The incidence of Streptococci (20%) obtained in this report was comparable with the results of David and Bonnier (1987). However, the incidence of Bacillus (3.9%) and Corynebacterium (1.46%) was low in the present study than reported by Jacob (1993).

Among the gram negative organisms cultured from the uterine fluids, E. coli (15.12%) was observed to be more predominant, followed by Pseudomonas (10.24%), Proteus (5.85%), Klebsiella (1.46%) and Citrobacter species (0.49%). This incidence was comparable with the results of Venkateswarlu et.at., (1993) and Ambrose and Pattabiraman (1993). However, Jacob (1993) reported a higher incidence of Citrobacter species (23.84%).

The incidence of mixed infection of 11.22 per cent recorded in this study was in line with the observations of David (1986). However, Venkaterwarlu et.al., (1983) and Ambrose and Pattabiraman (1993) reported higher percentage of mixed infections ranging from 20.37 to 33.76. The most commonly isolated organisms were Staphylococcus (30.24%) and Streptococcus (20.0%). On the whole, it was evident from this study that infection with gram positive organisms is 0.6 times higher than the incidence with gram negative organisms.

The results of antibiogram revealed that highest number of isolates were sensitive to Gentamicin (80.98%) followed by Nitrofurantoin (60.48%), Co-trimoxazole (51.75%), Chloramphenicol (50.73%);, kanamycin (45.85%), Ampicillin (28.29%) and Oxytetracycline (24.88%) in the descending order. Various authors have reported highly varying antibiotic sensitivity pattern of the uterine isolates (Bretzlaff et.al.

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1982; Sinha et.al. 1977 and Mulei and Gitau 1993). These differences might

be due to the variation in the number

of discs used and the resistance of the

organisms involved.

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# EFFICACY OF TREATMENT OF ENDOMETRITIS WITH CHEMOTHERAPEUTIC AGENTS IN COWS AND BUFFALOES.

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#### ABSTRACT

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Eighty nine crossbred cows and 15 buffaloes suffering from endometritis were treated with six different chemotherapeutic agents after bacteriological investigation and in-vitro antibiotic sensitivity test. The overall conception rates were found to be 63.85 and 53.85% in cows and buffaloes respectively. The conception rate in Lixen (Cephalexin) group was highest both in cows (69.23%) and buffaloes (67.67%).

Endometritis is one of the most common factors for reproductive failure in farm animals. Indiscriminate and prolong use of antimicrobial drugs in the absence of in-vitro sensitivity have contributed to the emergence of resistant strains of bacteria. Thus. isolation, identification and determination of drug sensitivity of the causative organism has become very important for effective therapy of Gynaecological infection and to limit the development of drug resistant strains (Dhami et.al., 1986, Maurya et. al. 1992, Gupta and Deoparker, 1993). Therefore, the present investigation was carried out to study the conception rate in treated animals suffering from endometritis.

#### MATERIALS AND METHODS

The experiment was conducted on 89 crossbred cows and 15 buffaloes taken from outdoor clinic and from well organised Khatals in and around Patna. These animals were selected on the basis of history, rectal examination and intermittent and continuous mucoprulent uterine discharge resembling first or second degree of endometritis.

Uterine samples were collected aseptically as per the method suggested by Dabas and Maurya (1988) with some modifications. Sensitively test for the bacteria was performed as per Bauer et.al. (1966). The organisms isolated were tested for in-vitro sensitivity and six chemotherapeutic agents were used. Cows were categorised into seven aroups, Group i (# 26) Lixen (Agrivet, India): Contained Cephalexin - 7.5% W/V equivalent to anhydrous cephalexin. Dose rate-10 gm powder + 20 ml distilled water. Group ii (# 19). Gentamicin (Karnataka antibiotics) contained Gentamicin sulphate, 40 mg, per ml. Dose rates 5 ml. + 15 ml.

distilled water. Group iii (# 14) Unimycin (Unichem) contained Neomycin sulphate 100 ml per ml (Neomycin base). Dose rate 5 ml + 15 ml distilled water. Group iv (# 9). Betalactin (Unichem) each two gram vial contained Ampicillin sodium equivalent to anhydrous Ampicillin 1 gm and Cloxacillin sodium equivalent to Cloxacillin 1 gm. Dose rate-1 vial + 20 ml distilled water, Group v (# 7) Vetclin (Sarabhai) : each gm contained 12 mg Tetracyclin hydrochloride. Dose rate-15 gm. + 20 ml. distilled water, Group vi (# 8) Sulprim - 24 (Unichem): each ml contained 200 mg sulphadiazine and 80 mg. Trimethoprim. Dose rate - 10 ml. + 10 ml. distilled water. Group vii (# 6) untreated control animals, Buffaloes were categorised into four groups Gr. i (# 6); Gr ii (# 4); Gr. iii (# 3) and Gr vii (#2) only.

The animals were treated with the drugs selected on the basis of sensitivity report. The drugs were given I/u alternate day in 6 doses. The animals were re-examined in subsequent estrus and inseminated with frozen semen. The response to treatment was assessed on the basis of pregnancy diagnosis between 45-65 days. The data thus obtained were statistically analysed as per Snedeor and Cochran (1967).

# **RESULTS AND DISCUSSION**

The conception rate among different treatment groups in cows ranged from 55.56 percent in Betalactin treated group iv to 69.23 percent in lixen treated group i The overall conception rate among various

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treated groups was 63.85%, however when considered individually the conception rate of Lixen treated group only was found to be significant (P < 0.05) Awasthi and Nema (1995), however, reported 90% conception rate which may be attributed to the study conducted on apparently healthy repeat breeder cows. Unimycin, Gentamicin, Sulprim-24 and Vetclin treated groups had 64.28, 63.15, 62.50 and 57.14 percent conception rate respectively. However, all these values were observed to be statistically nonsignificant which might be possibly due to small sample size. Sudhakar et.al. (1986) and Venkateswaralu et.al. (1983) have reported overall conception rates in cows to be 63% and 71.93% respectively.

The rate of conception in buffaloes was observed to be 66.67, 50.00 and 33.33% in Lixen, Gentamicin and Unimycin treated groups respectively. The overall conception rate was noted as 53.85%, however, the values were found to be nonsignificant. Chauhan and Tukker (1993) reported 62.50% conception rate in intrauterine antimicrobial therapy among buffaloes suffering from chronic endometritis.

#### ACKNOWLEDGEMENTS

The authors are thankful to Agrivet Farm Care Division, Glaxo India Limited. Agrivet, Unichem, Unichem Laboratories Ltd., Karnataka Antibiotics and Pharmaceuticals Ltd. and Sarabhai Chemicals for supplying free samples for the research work.

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MATERIALS AND METHOD

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Handling of semen and Artificial Insemination technique are two important factors for success of AI. Programme Trained to deposit fectinicians are trained to deposit semen at mid cervix or at the uncode of the Otenne body and the internal cervical on Minry women reported Indian J. Anim. Reprod., June, 2001; 22 : 60 - 62

EFFECT OF SITE OF SEMEN DEPOSITION ON CONCEPTION RATE IN ONGOLE COWS

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## ABSTRACT

A trial was conducted on 234 Ongole Cows having 40 to 120 days post partum interval to know the effect of site of semen deposition on conception rate. Animals were divided into three groups based on site of semen deposition. All the inseminations were done 10-12 hours following the detection of standing estrus with frozen semen (0.5 ml straw). 64 Cows were inseminated mid cervically (Group I), 83 Cows were inseminated into the body of uterus (Group II) and 87 cows were inseminated deep into the uterine horn (Group III) Ipsilateral to the impending ovulation. A Higher conception rate (73.63%) was observed with deep intra uterine horn insemination than the body of uterus (63.79%) and mid-cervical (53.68%) inseminations.

Handling of semen and Artificial Insemination technique are two important factors for success of AI Programme. Insemination technicians are trained to deposit semen at mid cervix or at the junction of the Uterine body and the internal cervical os. Many workers reported that the deposition of semen in the body of the uterus (Hunter 1984) or deep into the uterine horn ipsilateral to the ovary containing ovulatory follicle may increase the pregnancy rate (Pallares, et. al 1986; Senger, et. al. 1987 and Lopez-Gatius and camon-Urgel 1988). Hence the present study was undertaken in Ongole cows to know the effect of site of semen deposition in mid cervix, body of the uterus and deep into the uterine horn ipsilateral to the ovary containing the ovulatory follicle on conception rate. firs

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# MATERIALS AND METHODS

The study was conducted on 234 Ongole Cows having 40 to 120 days post partum interval maintained at Cattle project, Lamfarm., Guntur over a period of 18 months. The cows were free of Cystic ovarian follicles, abnormal genital discharge and of anatomical abnormalities of the reproductive tract detectable by rectal palpation. Cows in estrus were observed daily in the morning and evening by parading the vasectomised bull. All inseminations were performed by authors only 10-12 hours after the first observation of standing estrus, using frozen semen (0.5 ml straws) from one of seven proven Ongole bulls, which have high conception rate. Bulls were allotted proportionally to all the three groups of cows.

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In group I cows, semen was deposited in the mid cervix (64), in group II cows, semen was deposited in uterine body (83) and in group III cows semen was deposited deep into the uterine horn ipsilateral to the ovary containing the ovulatory follicle (87). The cows that returned to estrus after first insemination were bred again by depositing the same bull semen in the same site. Deposition of semen in the uterine horn ipsilateral to the ovary containing ovulatory follicle was confirmed by palpating the mature corpus luteum 10 days following the A.I. Pregnancy was confirmed by rectal palpation 60 days post insemination.

Chi. Square test as per the snedecor and Cochron (1967).

# **RESULTS AND DISCUSSION**

In group I out of 64 first and 31 second inseminations 33(51.56%) and 18(58.06%) cows became pregnant respectively with an overall conception rate of 53.68%. In group II out of 83 first and 33 second

#### Indian J. Anim. Reprod., June, 2001

insemination 50 (60.24% and 24(72.72%) cows were pregnant respectively with an overall conception rate of 63,79%. In group III out of 87 first and 23 second inseminations 64(73.56%) and 17 (73.91%) cows were conceived respectively with an overall conception rate of 73.63%. The difference in conception rates between the groups shows that the conception rate was significantly higher (p < 0.05) in cows with deep intra uterine horn inseminations than the mid-cervix inseminations, but nonsignificant with uterine body inseminations.

The results obtained from this study indicates that the deposition of semen deep into the Uterine horn ipsilateral to the side of impending ovulation influences the high conception rate in lactating Ongole Cows. This finding coincide with the findings of earlier workers, Fernandez Vancleve et. al. (1986). Zavos et.al (1985) and Lopez-Gatius and Camon-Urgel (1988) The higher conception rate in deep uterine horn inseminations might be due to the availability of more number of spermatozoa at the caudal portion of the oviduct as indicated by Lopez-Gatius and Camon-Urgel, (1988) since the cervical canal and uterine body do not act as a barrier to the transport of spermatozoa to the

site of functional reservoir sperm in the caudal portion of the oviduct.

Similarly high conception rate was observed in the present study by depositing the semen in the body of the uterus when compared to mid cervix but statistically nonsignificant, which was in agreement with the findings of earlier workers, Hunter (1984) and Momont et al. (1989). The lower conception rate in mid cervix inseminations might be due to loss of spermatozoa in the cervix as the frozen thawed spermatozoa are less capable of transit through the cervical barrier (Macpherson, 1968) leading to less number of spermatozoa in the oviduct where the fertilization occurs,

# ACKNOWLEDGEMENT

The authors are thankful to the Senior Scientist (AB), cattle project, L.R.S., Lamfarm, Guntur for allowing us to publish the data.

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Indian J. Anim. Reprod., June, 2001; 22:63-68

# QUANTIFICATION OF POSTNATAL CHANGES IN THE GERMINAL EPITHELIUM OF MADRAS RED RAM LAMBS MAINTAINED UNDER DIFFERENT FEEDING SYSTEMS

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#### ABSTRACT

Quantification of the germinal epithelium in Madras Red ram lambs during postnatal period was carried out. The number of supporting cells per cross section of seminiferous cords and the percentage of the seminiferous cords with gonocytes in the testis of Madras Red ram lambs were increased from birth to three months in pre-weaning group. The quantification of the germinal epithelium also revealed difference between grazing and feed lot systems. The percentage of most advanced stages of the seminiferous tubules were more in ram lambs of feed lot system than the grazing system.

The supporting cells present in the postnatal testis appeared to be the precursor of Sertoli cells (Skinner et.al., 1868). Monet-Kuntz et. al., (1984) reported that the large gonocytes gave rise to the germ cells.

Schanbacher et.al., (1974) stated that in a majority of the lamb's tubules only gonocytes and supporting cells were seen until about 60 days of age and spermatogonia appeared shortly thereafter. They have observed normal spermatogenesis in all the rams aged 180 days or older. Most of scientists the described the histological development of testis in male animals and they have not quantified the different cells present in the testis at different ages. Hence, the studies on quantification of germinal epithlium of Madras Red ram lambs during postnatal was carried out.

#### MATERIALS AND METHODS

Twenty six healthy Madras Red ram lambs born at Live-stock Research Station, Kattupakkam, Tamil Nadu were utilized for this study.

**Pre-weaning group :** Six ram lambs i.e., two ram lambs at birth, one month and three months of age were slaughtered and testes were collected for the present study.

**Post-Weaning group :** The remaining twenty ram lambs were weaned at 3 months of age. At the time of weaning, the ram lambs were equally and

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Associate Professor, Department of Animal Reproduction and Gynaecology, College of Veterinary Science, Tirupati.

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Part of Ph.D. thesis of the first author submitted to Tamil Nadu veterinary and Animal Sciences University, Chennai.

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randomly divided into two groups of different feeding systems namely grazing and feed lot systems. Ram lambs under grazing were sent for grazing daily. Concentrate feed at the rate of 100 g per lamb from 3 to 5 months of ages and 200 g per lamb from 5 to 8 months of age was fed in bulk for all the ram lambs as a supplement to grazing. Under feed lot system, each lamb was kept in separate wooden partitioned pens. They were fed individually ad libitum with pellet feed comprising of 50 per cent concentrate and 50 per cent lucerne meal from 3 to 8 months of age. Every month, two ram lambs from grazing and two ram lambs from feed lot systems were slaughtered from 4 to 8 months of age and testes were collected for the present study.

Immediately after slaughter, small tissue pieces of testis were cut and fixed in Bouin's solution for 24 hours. Adopting the standard methods described by Luna (1968) and Humason (1972), the tissues were processed for histological studies.

# Quantification of germinal epithelium

**1.Supporting cells and gonocytes :** From two section of each testis (Preweaning group) 100 seminiferous tubules were selected at random and studied for the presence or absence of gonocytes. The percentage of tubules containing gonocytes ranging from one to three and above were also estimated. The total number of supporting cells were counted at random from 20 tubules of each testis.

2. Germinal epithelial cells : The method described by Bilaspuri and Guraya (1986) for quantitative estimation of spermatogenesis was slightly modified to quantify the spermatogenesis in this study. Out of two sections from each testis of postweaning lambs, 100 seminiferous tubules were selected at random and studied for the association of the germinal epithelial cells. The tubules were classified into the following eight stages based on the association of the germinal epithelial cells.

Stage 1 : Tubules with prespermatogonia and spermatogonia.

Stage 2 : Tubules with spermatogonia.

Stage 3 : Tubules with leptotene or zygotene spermatocytes.

Stage 4 : Tubules with leptotene or zygotene and pachytene spermatocytes.

Stage 5 : Tubules with pachytene spermatocytes only.

Stage 6 : Tubules with round spermatics with one layer of spermatocytes.

Stage 7: Tubules with elongated spermatids with two layers of spermatocytes.

Stage 8 : Tubules with round and elongated spermatids with one layer of spermatocytes.

The percentage of incidence of each stage was estimated.

# **RESULTS AND DISCUSSION**

## Pre-weaning group

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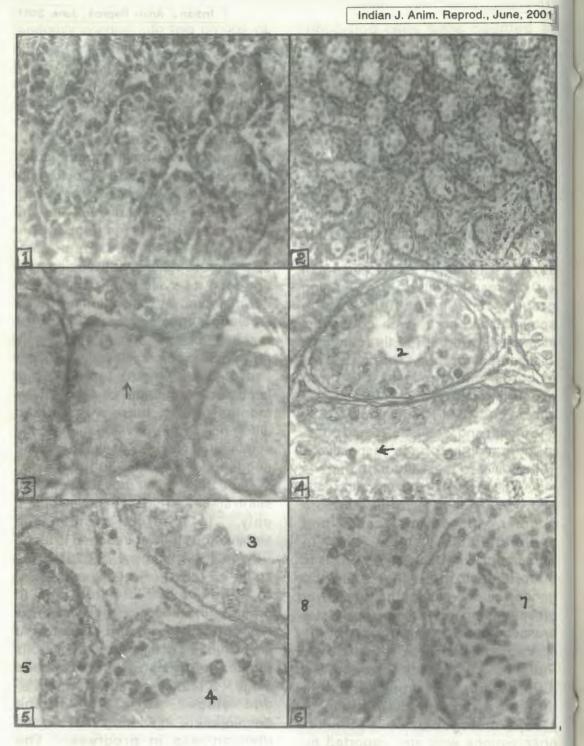
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The number of supporting cells per cross section of seminiferous cords in the testis of Madras Res ram lambs were increased from birth to three months (Table 1). The number of supporting cells at birth (Fig.1), one month and three months (Fig.2) were 12.09 + 0.13, 12.71 + 0.11 and 13.16 + 0.16 respectively in pre-weaning The percentage of the group. seminiferous cords with gonocytes increased from birth to three months in pre-weaning group. The percentage of cords with one, two and three gonocytes were 51.75, 15.25 and 3.00 at birth and 36.00, 27.75 and 27.75 at one month and 29.50, 38.75 and 30.25 at three months of age respectively. This indicated that after birth there is mitotic division of both supporting cells and gonocytes to give rise to more number of sertoli cells and spermatogonia which was in agreement with Galina (1972). It is interesting to note that while the gonocytes decreased, the number of prespermatogonia increased and later prespermatogonia was replaced by the spermatogonia. These results clearly indicate that the gonocytes differentiate into spermatogonia through an intermediate stage of prespermatogonia. Similar observations was also reported by Baishya et.al., (1987) in goats.

#### Post-weaning group

The percent incidence of different developmental stages of the seminiferous tubules in the testis of post-weaning ram lambs were presented in Table 2. The development of seminiferous tubules reached to stage 1 and 2 (Tubules with spermatogonia) only both in grazing and feed lot system ram lambs by the end of fifth month of age (Fig. 3). The difference in the development of seminiferous tubules between grazing and feed lot system ram lambs was not observed at four months of age. However, 90 per cent of tubules reached to stage - 2 in feed lot system ram lambs in contrast to only 76.75 per cent of tubules in grazing system ram lambs at five months of age. At six months of age, there was marked difference in the development of the seminiferous tubules between grazing and feed lot systems. In the grazing system, the seminiferous tubules were still in stage - 2 (Fig. 4) of development only. In feed lot system, the seminiferous tubules reached to stage - 2 (44.75%), stage 4 (27.25%) and stage - 5 (28%) of development (Fig. The meiotic division was in 5) progress in feed lot system which was not initiated in grazing system. At seven months of age, the seminiferous tubules in grazing system reached stage - 3 (42.5%), stage - 4 (24.5%) and stage - 5 (33%) of the development indicating the meiotic division was in progress. The seminiferous tubules in feed lot system



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# TABLE 1

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# Quantification of supporting cells and gonocytes in seminiferous cords of ram lambs in pre-weaning group

THENELOUS	Age				
Parameter	Birth	1 Month	3 Months		
No. of supporting cells per seminiferous cord	12.09+0.13	12.71+0.11	13.16+0.14		
Percentage of seminiferous cords with supporting cells and one gonocyte	51.75	36.00	29.50		
Percentage of seminiferous cords with supporting cells and two gonocytes	15.25	27.75	38.75		
Percentage of seminiferous cords with supporting cells and three and more gonocytes	3.00	27.75	30.25		
Percentage of seminiferous cords with supporting cells only	30.00	8.50	1.50		

# TABLE 2

# Quantification of germinal epithelium in ram lambs of post-weaning group

Feeding	Age	Percentage of different developmental stages of seminiferous tubules							
System	(Months)	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Stage 7	Stage8
	4	70.75	29.25			-	_	-	-
	5	23.25	76.75	-	-	-		-	
Grazing system	6 .	6.50	93.50	-	-		-	-	-
	7	-	-	42.50	24.50	33.00	-	-	-
	8	-		-	7.25	3.50	42.50	28.50	18.25
Feedlot system	4	63.50	36.50	-		-	-	-	
BOR AD ALD	5	10.00	90.00	-	11.21	10 1	-	-	-
	6	-	-	44.75	27.25	28.00	-	-	-
	7	-	-	1 61 2 1	-	-	39.25	33.00	27.75
	8	-	-		-	-	30.25	36.50	33.25

reached stage - 6 (39.25%), stage - 7 \_ \_ be at stage - 4 (7.25%) and stage - 5 (33%) and stage - 8 (27.75%) at seven months of age and the spermatogenic wave was established (Fig. 6) On the contrary in grazing system at eight months of age only, the seminiferous tubules reached the last stage of development i.e. stage - 6 (42.5%), stage - 7 (28.5%) and stage - 8 (18.25%). Even at 8 months of age some of the tubules of ram lambs kept under the grazing system continued to

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(3.5%) of development indicating that the spermatogenic wave has not been established in all the tubules.

#### ACKNOWLEDGEMENT

The financial assistance provided by I.C.A.R., New Delhi and A.P. Agricultural University, Hyderabad for Ph.D. programme is thankful by acknowledged by the first author.

### **LEGEND FOR FIGURES IN PAGE 66**

- Fig. (1) at birth-Note the centrally located gonocytes and peripheral supporting cells.
- Fig. (2) at 3 months Note the prespermatogonia;
- Fig. (3) at 5 months Feedlot Note the fragmentation of the luminal cytoplasm (arrow) and prespermatogonia and spermatogonia (Stage 1)
- Fig. (4) at 6 months Grazing Note the lumen at the centre of the tubule and spermatogonia along the basement membrane (Stage 2)
- Fig. (5) at 6 months Feedlot Note the well developmed lumen and spermatocytes (Stages 3, 4 and 5)
- Fig. (6) at 7 months Feedlot Note the well established spermatogenesis.

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TESTICULAR BIOMETRY AND SPERM PRODUCTION IN HOLSTEIN FRIESIAN AND CROSSBRED BULLS\*

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#### ABSTRACT

This investigation was undertaken on 11 healthy breeding bulls (4 HF; 4 K x HF; 3 K x J) of 4-6 years age. The assessment of scrotal circumference. testicular dimensions and semen quality was made at monthly interval over a period of 10 months. The mean values of scrotal circumference and testicular measurements (Length, width, thickness) were found to be significantly greater in HF than the crossbred bulls (40.42; 15.53; 9.47 and 8.45 cm, respectively), yet the seminal attributes did not differ significantly between them, except the individual sperm motility which was lower in K x J (83.67 ± 1.09%) than in K x HF (86.50 ± 54%) HF (87.75 ± 1.05%) bulls. Further, the length, width and thickness of left testes were significantly higher as compared to right testes in all the three genetic groups. Scrotal circumference had positive high and non-significant correlations with the reaction time and the

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seminal attributes. The reaction time had significant positive correlation only with semen volume, whereas all other correlations among the seminal attributes were positive but insignificant.

Evaluation of breeding soundness in any breed requires an accurate knowledge about the normal testicular size and semen characteristics in that particular breed, since these traits vary in different breeds and species. Reports are scanty on biometry of testes and seminal characteristics in purebred HF and its crosses reared in tropics (Rao and Rao, 1980; Patel et al., 1989). The present study was, therefore, attempted to study andrological and semenological aspects of pure HF and crossbred bulls used in breeding programmes of Gujarat State.

#### MATERIALS AND METHODS

Eleven sexually matured healthy breeding bulls of three genetic groups (4 HF; 4 K x HF; 3 K x J), aged 4-6 years, stationed at Central Sperm Station, Jagudan, Mehsana, Gujarat, formed the material for this study. The bulls were maintained under identical

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nutritional and managerial conditions and were under a regular weekly twice semen collection schedule through out the year. For the present work, ten observations each on scrotal biometry and semen quality assessment were made at monthly intervals on each bull. The clinical investigation of bulls including measurement of scrotal circumference, testicular length, width and thickness were made as per Rosenberger (1979). Semen ejaculates collected in artificial vagina evaluated were macro-and microscopically using standard techniques. The data were analysed statistically to know the breed differences, if any, in the above traits.

# **RESULTS AND DISCUSSION**

The detailed andrological examination of bulls revealed that the breed had no influence on the type of preputial sheath (tight, medium or pendulous) or the shape of scrotal sac (square, elongated or oblong) in the breeding bulls. It is known that these traits have no relation to fertility in bulls (Rao and Rao, 1988). However, semen collection is easier in bulls with tight or medium sheath. The reaction time and service behaviour were found to be satisfactory in all the bulls under study without breed difference and agreed with the reports of Patel (1985) and Patel et al. (1989). Seminal vesicles were also found to be normal in size and shape on rectal examination in all the bulls.

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The findings on the scrotal biometry (Table) revealed that the HF bulls had significantly greater scrotal circumference as well as testicular length, width and thickness as compared to K x J and K x HF bulls. Insignificant differences were also observed in these traits between bulls within the group (Table), the mean length, width and thickness of left testes was significantly higher than the right testes in all the three genetic groups. These results compared well with those of Patel (1985), Singh and Pangawkar (1989), Luthra and Marinov (1991) and Veerapandian et al (1992). The scrotal circumference of the bulls is an indirect indication of the number and size of seminiferous tubules in testes of the bull, and thus, it gives an idea about the sperm output in breeding bulls (Coulter and Foot. 1976). The present findings reveal that the potential of sperm production in HF bulls is significantly more than the crossbreds. These results are also in agreement with those of Singh and Pangawkar (1989).

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Further, the seminal attributes give a fairly good analysis about the breeding protential of the male animals. In the present study the ejaculate volume of the HF bulls did not differ significantly from that of K x HF and K x J bulls, even though HFs had greater scrotal circumference and testicular size, These results compared well with those of Tuli et al. (1988). However, Rao and Rao (1980), Bhosrekar et al. (1985),

ter tri anexastra hats. Prod y	K x HF (4)	K x J (3)	(4)
Scortal circumference (cm)	35.22 ± 1.69	35.78 ± 0.94	40.42 ± 2.49
Testicular length (cm)	18-58 11		burn and
Right	13.41 ± 0.36	13.72 ± 0.36	15.53 ± 0.54
Left	12.33 ± 0.30	12.63 ± 0.44	14.60 ± 0.64
Testicular width (cm)			
Right	8.61 ± 0.41	8.42 ± 0.51	9.41 ± 0.28
Left	7.87 ± 0.39	7.70 ± 0.51	8.77 ± 0.26
Testicular Thickness (cm)	the second se	THE A DURLAN	to annear he must
Right	7.65 ± 0.29	7.65 ± 0.28	8.45 ± 0.26
Left	6.86 ± 0.26	6.77 ± 0.26	7.90 ± 0.26
Ejaculate volumn (ml)	5.05 ± 0.98	4.28 ± 0.23	4.59 ± 79.12
Mass motility (0-5)	3.93 ± 0.14	3.87 ± 0.20	3.98 ± 0.13
Sperm Concentration (10 <sup>6</sup> /ml) Individual motility (%)	1583.00 ± 72.28 86.50 ± 0.54	1610 ± 99.19 83.67 ± 1.09	1670.75 ± 79.12 87.75 ± 1.05
Live Sperm (%)	90.93 ± 1.94	92.0 ± 2.15	92.18 ± 0.90

TABLE : Scrotal circumference, testicular dimension and seminal attributes in breeding buls of different breeds (Mean ± SE)

Belorkar et al. (1990) and Singh and Pangawkar (1990) reported significant breed differences in the semen volume.

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The mass activity and individual motility of the spermatozoa was found to be superior in HF bulls than the crossbreds under study. Yet the difference was significant only in case of individual sperm motility (Table) These findings are in agreement with those of Patel (1985) and Singh and Pangawkar (1990). The sperm concentration and the percentage the live spermatozoa were also non-significantly higher in HF than the crossbred bulls. Singh and Pangawkar (1990), however, observed significant breed variations in these traits.

In the present study, the correlations of scrotal circumference with seminal attributes were highly positive but nonsignificant. Reaction time was, however, significantly and positively correlated with ejaculate volume. All the other inter relationships between seminal attributes were low and positive. These findings compared well with those of Patel (1985) and Belorkar et al. (1990).

## ACKNOWLEDGEMENTS

The senior author is grateful to the Directorates of Animal Husbandry, Gujarat State, Ahmedabad, and CSIR, New Delhi for grant of deputation financial assistance in the form of SRF for his doctorate studies. The facilities and cooperation extended by the management of Dudhsagar Dairy, Mehsana for taking up this work at their Sperm Station is also acknowledged.

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INDUCTION OF ESTRUS IN DELAYED PUBERTAL CROSS-BRED HEIFERS WITH RECEPTAL AND LONG ACTING STEROID PREPARATION.

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One of the major reproductive problems in dairying is delayed puberty. The present experiment was undertaken to evaluate the efficacy of receptal and a combination of longacting sex-steroid in anestrus delayed pubertal heifers and to monitor effect of these treatment on certain blood constituents.

### MATERIALS AND METHODS

Cross-bread heifers which attained adequate body weight (over 200 kg) and age (over 2 years), but failed to exhibit estrus and had smooth ovaries and atonic uterus were regarded as delayed pubertal heifers. Such twenty one heifers were randomly divided into three groups and were assigned to treatment as below

**Group I :** Treatment with combination of 5 mg of estradial valerate (Progynon Depot) and 50 mg of hydroxy progesterone caproate (Proluton Depot, German Remedies Ltd.) intramuscularly per animal in castor oil.

Group II: Treated with 5 ml. receptal (Buserelin acetate 0.0042 mg/ml) intramuscularly.

Group III : Untreated anestrous cross-

bred heifers were served as control.

The experimental animals were maintained under uniform managemental conditions. Estrus detection was done by the teaser bull, and those detected in estrus were served naturally. Ovulation was confirmed by detecting corpus luteum on 12th and pregnancy diagnosis on 60th day post-service by rectal palpation.

Blood samples were collected from all the experimental animals on the day of treatment and at induced estrus or 30 days post-treatment in non-responding cases. The blood glucose was estimated by Folin and Wu (1920), inorganic phosphorus by Fiske and Subbaraw (1925) and calcium by Clark and Colip (1925).

## **RESULTS AND DISCUSSION**

The comparative efficacy of the two remedial measures versus control for induction of estrus and conception rate presented in Table-1

In the present study, the combination of long-acting steroids gave excellent results with predictable estrus and high fertility. Finding

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 Table 1: Comparison of treatments in relation to the duration of estrus induction, estrus pattern and conception rate.

Group	Treatment (No. of Animals)	Treatment to estrus induction duration (in days)	MA FIU	Conception			
			Intense	Inter mediate	Weak	Absent	Conception- rate
- Pathesa	Progynam Depot + Prolution Depot (7)	7.57 + 1.23	3	4	n nellem En let	e pitthe	71.42
matim	Receptal (7)	23.50 + 1.98	2	.105mh	ng ka Ju ng ka Ju	ang has	66.66
III	Control (7)	a action and	-gn -gn	al io ac sistered	inblnati nanasi	thoreas	acting sex

corroborates with reports of earlier workers (Agrawal and Patil, 1983; Shankar et al., 1988).

The results obtained with receptal are good but the induction of estrus and conception rate are slightly lower than combination of long-acting steroids. However, the success rate with receptal closely in agreement with earlier reports (Thakur et at., 1996; Sankhyan, 1992). Both the treatment had no significant difference in causing changes in blood levels of glucose, calcium and inorganic phosphorus and no correlation of their levels with either treatment could be drawn. Thus it can be concluded that treatments are quite efficacious in inducing estrus in delayed pubertal heifers, however steroid combination proved better in shortening induction time and improving fertility.

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# EFFECT OF YEAR, GENETIC, HEIFER AND COW GROUPS ON CONCEPTION RATE IN CROSSBREDS

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Conception rate (CR) determines the fertility status of breedable population in the herd. Improved conception rate minimizes the maintenance cost of the herd, and increases the reproductive life of the female. To know the effect of year, genetic group, heifer and cow group on conception rate present investigation was undertaken.

### MATERIALS AND METHODS

Data on conception rate in different genetic groups was collected from All India Co-ordinated Research Project on Cattle and Research cum Development Project on Cattle of this University, for period April, 1986 to March, 1997. All animals were kept under identical conditions of feeding, breeding, and management. Artificial inseminations were carried out by liquid semen of the farm bred breeding

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bulls. Pregnancy diagnosis or confirmation of conception was done by per rectal examination 60 days after insemination. Conception rate was calculated by the following formula-

Total number of animals conceived CR = \_\_\_\_\_\_ x 100 Total number of inseminations required

In HG group (50% Holstein -Friesan + 50% Gir) 2594, in FJG group (50% Holstein Friesan, + 25% Jersey + 25% Giri) 4353, in JFG group (50% Jersey + 25% Holstein Friesan + 25% Gir) 143, in BFG group (50% Brown Swiss, + 25% Holstein Friesan, + 25% Gir) 1740, inseminations were carried out. In Heifer and Cow group, 2662 and 6168 inseminations were carried out. In all 8830 inseminations were under taken on the farm during the study period.

### **RESULTS AND DISCUSSIONS**

On the farm 2594, 4353, 143, 1740, inseminations were undertaken, from which 660, 1343, 35, and 542 cows were conceived leading to 25.67%, 30.85%, 24.47% land 31.41% conception rate, in HG, FJG, JFG, and BFG genetic groups, respectively. Total 8830 inseminations were carried out, from which 2580 cows were conceived, leading to overall conception rate of 29.29%. Conception rate, in BFG and FJG was more or less similar, while less conception rate in JFG group might be due to small number of observations due to changed breeding policy and elimination of the same genetic group from the herd.

Less conception rate in HG group might be due to inheritance of indigenous Gir blood which is well known for repeat breeding and infertility problems. (Anonymous, 1986). In the present study no significant (p> 0.05) variation in conception rate in different genetic group were observed, which is in agreement with the observations made by Belsare and Deshmukh (1992).

These studies revealed that, conception rate in heifers (28.66%) was less as compared to the cows, but no statistically significant difference was noted in these two groups, which is in agreement with Ulmek and Patel (1992). This might be due to age

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factor as the adults are expressing full blooming heat due to adequate development or reproductive organs, while heifers are showing silent heat due to hypoplasia of genital organs, malnutrition and managemental negligence because more attention is paid to the lactating animals only. In heifer and cow group the lowest conception rate 25.21% and 19.67% were recorded for which six and more inseminations were required forconception. The highest conception rate 29.83% and 35.66% were recorded for heifer and cow group, for which four and one inseminations were required for conception, respectively. When the data were analysed year wise the highest and the lowest conception rate were observed in the year 1990-91, and 1996-97 respectively. The lowest conception rate in the year 1996-97 might be due to economic crises leading to poor nutritional status of the breeding stocks. In present studies, 27.0% cows conceived after 1st, 32.53% after IInd, 32.21% after IIIrd, 30.13% on IVth, 25.34% on Vth and 20.47% on VIth or more inseminations.

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## PHYSICAL CHARACTERISTICS OF UDDER SECRETIONS DURING LAST TWO WEEKS OF GESTATION IN COWS

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External changes in vagina and pelvic ligaments during advanced gestation are considered to be the signs of approaching parturitions in cattle, while the udder secretions prior to parturition change from honey-like dry secretion to yellow turbid, opaque secretion, referred to as colostrum (Roberts, 1971). The present study was carried out to see, whether the changes in udder secretions corroborate with above pattern and if these could be considered as an indicator of parturition.

## MATERIALS AND METHODS

Udder secretions from cows (Jersey and Jersey x Red Sindhi n=80) of different reproductive parity were collected to record colour and viscosity, beginning with 2 weeks before parturition. Of the 80 cows evaluated, observation could be recorded only in 49 animals for the entire study period, since the remaining animals had either blood and / or pus mixed secretions or there was a variation in the secretions between different teats of same animal.

## **RESULTS AND DISCUSSION**

Changes in appearance of udder secretions during last two weeks of gestation have been presented in the Table.

Straw-yellow and highly viscous (honey): Between days 14-11 majority of the animals (~ 60%) exhibited this form of secretion, while on the day calving approximately 12 % of the animals had this secretion.

Straw-yellow and moderately viscous: During the course of study 11-17 animals exhibited this form of secretion.

Mild yellow and mild viscous (colostrum): With the passage of time, there was gradual increase in the number of animals exhibiting this type of secretion. But a day before calving, majority of the animals (~ 50%) had colostrum like secretion.

**Milk:** At the start of study only one animal had milk in udder, which was most probably because of a limited dry period of 2 weeks. High milk yield potential could be another cause of prepartum presence of milk (Roy, 1990)

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In majority of the cows, mammary secretions changed from honey to colostrum like secretions prior to calving which is in concurrence with Roberts (1971). In other animals, variation may be because of a dam effect and management associated factors (Roy, 1990). In addition, failure of similar secretions between different teats of same animal (n=24) may render this criteria unsuitable for indicating ensuing parturition.

Table: P	ercentage of	Incidence	of Cows	s with differ	ent udder	secretions
	durir	ng last 2 w	eeks of	Gestation (r	1=49).	

Characteristics	Days to calve					
(Color and Viscosity)	11-14	8-10	5-7	2-4	1	
Straw-yellow & highly viscous (honey)	60	43	44	32	12 00	
Straw-yellow and moderately viscous	26	39	23	33	28	
Mild yellow and of mild viscosity (colostrum)	12	18	25	26	50	
Milk	2	4	8	9	10	

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## EFFECT OF ANTIBIOTICS ON THE BACTERIAL LOAD AND QUALITY OF SEMEN OF MURRAH BUFFALO BULLS DURING PRESERVATION

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On preliminary in-vitre sensitivity study it was observed that Amikacin (89.71%), Norflexacin (88.24%) and Gentamicin (85.29%) were most effective antibiotics while the routinely used Streptomycin and Penicillin were effective against 67.65 23.53 per cent isolates respectively. The present study is to assess the effect of these antibiotics on the bacterial load and semen quality of Buffalo Bull during preservation.

## MATERIALS AND METHODS

A total of 24 ejaculates consisting of 6 from each of 4 Murrah buffalo bulls were used for this study. Immediately after collection 13 split samples of the semen were extended (1:25) in Tris extender (Davis et al 1963) containing different levels of antibiotics and then preserved at refrigeration temperature upto 48 hours. The dose of antibiotics per ml of extender were Penicillin 800 IU + Streptomycin 1000/ug, Gentamicin 500/ug, Amikacin or Norflexacin (100,250,500,1000 or 2000/ug). The extended semen was evaluated for bacterial load, progressive motility and live sperm count at 0, 24 and 48 hours of preservation. The bacterial lead was determined by standard surface viable count method of Cruickshank et al. (1975) on blood agar plates. The progressively motile sperm were estimated by conventional methods and the live sperm were estimated by differential staining technique using eosine nigresin stain (Campbell et at., 1953).

### **RESULTS AND DISCUSSION**

The analysis of variance revealed that the variation in mean percentage of motility, live sperm and mean bacterial load was highly significant between bulls, between treatments and between preservation periods. The percentage of motility and live sperm varied significantly due to treatment x preservation and x bull interaction. A non significant negative correlation was found between sperm motility and bacterial load (-0.223) and live sperm and bacterial load (-0.228)

The overall highest motility of spermatezea in semen extended with

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different levels of Amikacin differed significantly from that recorded for Gentamicin but did not differ significantly from that recorded for different levels of Norflexacin. The present findings in respect of superiority of Amikacin in maintaining the motility during preservation are in close conformity with the earlier workers (Arriola and Foote, 1982). The significant reduction of motility observed in Gentamicin treated semen samples supports the finding of Ahmed and Foote, (1984); Jasake et al (1993) while centradicts the results Indian J. Anim. Reprod., June, 2001

of Farooq et al. (1993). Norflexacin upto 2000/ug can safely be added to extended semen to control the bacteria without affecting the semen quality. Better efficacy of Amikacin, Norflexacin and Gentamicin observed in the present study against different organism present in semen are in agreement with earlier workers (Wayda, 1991 and Ramaswamy et al., 1994). Better efficacy of specific antibiotics was observed as these antibiotics were selected based on sensitivity test.

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## EFFECT OF DIFFERENT ANTIBIOTICS ON SEMEN QUALITY DURING POST THAW INCUBATION AT 37°C

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The present investigation was undertaken to find out the effect of different antibiotics on motility and acresomal integrity of buffalo bull sperm in tris extender during post thaw incubation at 37°c.

A total of 24 ejaculates consisting of 6 from each of 4 Murrah buffalo bulls were used for this study. Immediately after collection 5 split samples of the semen were extended (1:25) in tris extender (Davis et al., 1963) containing different antibiotics. The dose of antibiotics per ml of extender were Pencillin 800 iu + Streptomycin 1000/ug, Gentamicin 500/ug, Norflexacin 500/ug Amikacin 500ug and control without antibiotics. The diluted semen samples were frozen in french medium straw in the liquid nitrozen vapour and stored in liquid nitrozen for 24 hours. The frozen straws were thawed in a water bath at 37°C for 30 seconds were kept in an incubator at 37°C for 4 hours.

 Dept. of Gynaecology, C.V.Sc.. Guwahati -781 022, Assam The motility was assessed just after thawing and after 1,2,3 and 4 hours of incubation by conventional methods. The acrosomal integrity was studied just after thawing and after 1 and 4 hour of incubation using Giemsa stain according to the methods of Watsen (1975).

The percentage of progressively motile sperm 55.00 ±  $1.01, 27.19 \pm 1.04, 38 \pm 0.98, 9.17 \pm$ 0.96 and  $3.50 \pm 1.02$  after thawing and after 1,2,3 and 4 hours of incubation respectively was significantly higher in semens containing Amikacin than any other antibiotics after thawing and different hours of incubation. This is in agreement with Arriola and Foote (1982). The mean percentage of motile sperm and intact acrosome in semen extended with different antibiotics were significantly higher than no antibiotics at each period of incubation. It is concluded that Ami kacin is the best antibiotic to maintain optimum motility and acrosomal integrity in extended Buffalo semen.

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## INFLUENCE OF TUBECTOMIZED EWES IN EXPRESSION OF SEXUAL BEHAVIOUR OF RAMS REARED IN UNISEXUAL FLOCK

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The objective of this study was to evaluate to effect of short exposure of oestrus ewes on the sexual behaviour of rams.

## MATERIALS AND METHODS

Fifty Chokla rams of more than one year of age were randomized into two groups (Group A Group B) and were kept separately under routine farm management practice. Only experimental group of 28 animals (Group A) had an access of tubectomized oestrus ewes twice a week during night shelter for a period of 45 days. Ewes were brought into estrus by the injection of stilboesterol 24 h prior to exposure to male flock. **Observations on different sexual** behaviour were recorded by exposing each ram of both the groups to an oestrus female.

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# RESULTS AND DISCUSSION

Rams exposed to females (Group A) exhibited more mounts and ejaulations with less reaction time (10.5 sec) than the control (13.5 sec). From Group A 3.5% of the rams were sexually inactive as compared to 13.6% in Group B. Rearing of rams in unisexual flocks after weaning in isolation led to homosexual observation were prominent at the initiation (0 day) of the experiment in both the groups. Although the period of the study was short but there was a marked improvement in rams in terms of sniffing genetalia and their sexual interest in the oestrus ewe which is evident from the low number of animals having no interest in the ewe as compared to the high incidence in rams prior to the initiation of this study. It was also observed by Srivastava et al (1989) and Katz et al (1988) that rams reared with females are significantly better than that rams reared without females.

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## INFLUENCE OF PLASMA PROGESTERONE LEVEL AND CERVICAL MUCUS FERN PATTERN AT OESTRUS ON CONCEPTION RATE IN BOVINES

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It is essential that the insemination should be done at the optimum time to obtain higher conception rates. Many workers reported that conception rates were higher in bovines when inseminations were carried out at low progesterone levels (Appleyard and Cook, 1976; Antal et al., 1987). However, the cost and time involved in the estimation of progesterone limits its use in routine AL. Hence, any other simple parameter which reflects the level of progesterone can be considered for deciding the proper time of insemination. Cervical mucus shows clinical changes in its physical and chemical properties, which are reported to be under the control gonadal hormones (Eltohamy et al., 1990). Hence, the present study was undertaken to assess the relationship between the plasma progesterone level at the time of insemination and fern pattern of the cervical mucus and their influence on conception rate in cattle and buffaloes.

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## MATERIALS AND METHODS

Apparently healthy animals that were brought for AI (70 cattle and 69 buffaloes) were utilized for this study and they were inseminated with frozen semen. Blood samples were collected at the time of insemination in heparinized vials (Heparin 10 IU/ml blood). Plasma was separated and stored at - 20° C until analysis.

Cervical mucus was collected at the time of insemination and fern pattern was assessed as done by Sukj Deo and Roy (1971). Progesterone was estimated by using Radio Immunossay with COAT-A-Count (Progesterone, DPC, Los Angeles) as per the procedure given by the manufacturers.

Pregnancy verifications were carried out 45 days post insemination by rectal palpation. Conception rate was calculated based on 1st insemination. Statistical analysis was done using the standard methods of Snedecor and Cochran (1989).

## **RESULTS AND DISCUSSION**

Of the 70 cattle, 33, 28 and 9 showed typical, atypical and nil type fern pattern. The CR was 63.63 and

Part of M.V.Sc. thesis submitted by the first author to the Deemed University, IVRI, Izatnagar 243 122.

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21.42% in cattle inseminated when their cervical mucus showed typical and atypical fern pattern respectively. Out of 69 buffaloes, 40,16 and 13 showed typical, atypical and nil fern pattern. CR of 57.50 and 18.57% were obtained in buffaloes with typical and atypical fern pattern. Significantly higher CR was obtained in both cattle and buffaloes with typical fern pattern and no pregnancy was obtained with nil fern pattern. These results are in agreement with those of Agarwal and Purbey (1983) and Dhailwal and Sharma (1988). They reported a higher CR in animals inseminated at typical fern pattern.

The CR was higher in cattle (69.23%) and buffaloes (68%)

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inseminated when both progesterone levels were low and fern pattern was typical. It was found that majority of the animals showed typical fern pattern when the progesterone level was <0.1 ng/ml. When the progesterone level increases the fern pattern also change from typical to atypical and then to nil.

The reason for no pregnancy in animals inseminated at nil fern pattern which reflects high  $P_4$  levels may be due to unfavourable environment provided to the sperm. It has been previously reported that the luteal phase cervical mucus, when the progesterone is high, prevents sperm transport (Eltohamy et al., 1990) resulting in impaired fertility.

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## TOTAL PROTEIN CONCENTRATIONS IN UTERINE SECRETIONS OF PREGNANT AND NON-PREGNANT GOATS

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The uterine secretion constituting the local environment of the early embryo, is important in mammalian reproduction, particularly in domestic ruminants in which the processes of implantation and placentation are relatively slow (Greenstein et.al., 1958). A few uterine secretory proteins have even been purified or characterized (Bazer et al 1981). The present studies were undertaken to estimate the concentration of total protein in uterine secretions of pregnant goats.

### MATERIALS AND METHODS

Uterine flushing were collected from the uteri of 12 nonpregnant and 8 pregnant goats, slaughtered in early stages of pregnancy at the local abattoir, by flushing the uteri with PBS and the levels of total proteins in the uterine secretions were analysed. The method for estimation of total proteins reported by Reinhold (1953) was followed with minor modifications.

### **RESULTS AND DISCUSSION**

The mean value of total proteins was 0.096+0.035 g/100 ml in case of non-pregnant goats and 0.119 + 0.043 g/100 ml in case of pregnant goats. The difference in values between pregnant and non-pregnant goats was not significant.

More studies on a larger sample size, samples from various stages of pregnancy and various proteins in different stages of pregnancy may provide further information on stage-specific expression and possible functions of such proteins.

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# SEASONAL VARIATION IN THE REPRODUCTIVE EFFICIENCY SAHIWAL COWS.

## M. PRASAD, R.P. TIWARI AND C. SINGH

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Season was reported to influence the reproductive efficiency of cows (Grewal et.al., 1974 and Kaushik et.al., 1979. The effects of season on the conception rate of Sahiwal cows maintained in a Farm is studied and results are analysed.

## MATERIALS AND METHODS

Three hundred fourteen successive services covering 25 years breeding records of Sahiwal cows maintained at Dairy farm of C.S. Azad University of Agriculture and Technology Kanpur between the years 1971-95 were used to collect the data. The observations on the number of services and conception rate were collected from history sheets of Sahiwal Cows and only those records which had complete information were Month and seasonal utilised. (Summer-June 15 August; Autumn -September to November; Winter -December to February; and Spring March to May) variation in number of services and conception rate was studied and subjected to satistical analysis. (Snedecor and Cochren, 1968).

# **RESULTS AND DISCUSSION**

Distribution of number and

percentage of services in different months of the year revealed that the indices of conception are uniform in all the months of the year, the highest percentage (12.4) of conception was recorded in the month of March whereas it was lowest (3.5) in the month of August. Maximum number of conception in Sahiwal cows was mostly confirmed in the month of March (12.4) April (10.5) May (10.1) October (9.5) and November (9.5).

Based on unequal number of observation a non significant difference of months on breeding efficiency was found. In respect of breeding efficiency the month of March was superior to other months. December month showed the poorest breeding efficiency as compared to all other month of the year. Morgan and Davis (1938) reported highest fertility in December and lowest in September. Erb (1942) observed the highest fertility in May and it was lowest in August. These findings are in close agreement with present investigation. Burgess et.al (1953) studied the effect of month upon breeding efficiency and found that there is no significant differences between the month as regards breeding efficiency of cows.

As regards effect of season upon breeding efficiency it was evident that the spring season was the best season among all seasons for the conception. It was highest (32.85) in spring season and lowest (17.68) in autumn season. In summer and winter the conception rate was 31.21 and 23.10 respectively. The seasonal variation in breeding efficiency of Sahiwal cows found to be highly significant.

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Singh and Pandey (1970) reported non significant difference between seasons on the breeding efficiency of the cows. Grewal et.al (1974) reported that the month and season of insemination had no effect on the conception rate in Hariana cows

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which was found to be in close agreement with present study. Tiwari and Kushwaha (1982) studied higher reproductive efficiency in the winter and spring season in Sahiwal cows. But in case of buffaloes winter and rainy seasons had higher reproductive efficiency, Chaurasia et.al. (1987) and Methekar et.al. (1992) reported that the breeding efficiency was not significantly affected by the season where as Tailor and Jain (1992) reported that winter was most favourable and summer was unfavourable season for conception rate in buffaloes. It was concluded that seasonal difference was highly significant for the conception. Spring season comprising of March, April and May was superior to other seasons.

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### REPRODUCTIVE TRAITS IN PATANWADI EWES AND ITS CROSSES

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eviduo In the recent past, the ewe has become the most preferred animal for research on mammalian reproduction. It is imperative that the reproductive traits of the ewe be understood in order to carry out successful research. Physiological processes like pregnancy and parturition are a co-ordinated series of highly complex nature, which needs to be well understood and well managed. If the average gestation length, birth weight and the probable time of lambing on a farm is know, the management would be greatly facilitated. Keeping this in mind, the present study was conducted in Patandwadi and its crosses. maintained at Livestock Research Station, Gujarat Agricultural University, Sardarkrushinagar.

### MATERIALS AND METHODS

The reproductive traits were

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noted in 81 ewes of the native Patanwadi (P) breed and its crosses with Merino (MP) and Rambouillet (RP, 5RP and 3 RP). Length of gestation, weight of lamb at birth, time of lambing and rate of twinning and sex ratio at birth were recorded. Length of gestation of each artificially inseminated ewe was calculated from the day of successful AI to the day of lambing. Immediately after birth, cleaned and dried lambs were weighed using standard balances. Twenty four hours period of the day was divided into eight equal intervals viz., 0.01 to 3.00, 3.01 to 6.00 hrs. and so on, and the lambing during these intervals were recorded. The number of twins born to the ewes in the flock were also recorded.

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### **RESULTS AND DISCUSSION**

Length of gestation : The average gestation length (days) was 149.49 + 0.23, which was in the range reported by Toteda et al (1988), Trimnell et al. (1988) and Sahni and Lalchand (1990). The average minimum and maximum gestation lengths was noted in 3 RP and in MP breeds respectively. However, no significant difference was found between the breeds under study for gestation length, which were in line

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with the findings of Sahni and Lalchand (1990).

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The average gestation length (days) for the male lamb (150.12 + 0.32) was found to be significantly (p < 0.01) higher than that for the female lamb (148.85 + 0.30). The findings were similar to those reported by Mail et al. (1985) and Musci and Turi (1988) but differed from that reported by Toteda et al. (1988), which could be due to the different breeds studied.

In the only case of twin lambing (mixed sex twins) in RP breed, the gestation length was found to be 151.00 days.

Weight of lamb at birth : The average weight (kg) of male lambs, born to the ewes under study, was 3.18 + 0.09 and that for female lambs was 3.03 + 0.09. The minimum and maximum birth weights for male lambs were found in 3 RP and in MP breeds, whereas in female lambs it was observed in 3 RP and P breeds, respectively. The average sexwise birth weight of male (3.18 + 0.09) and female (3.02 + 0.09)lambs found here were lower than those reported by Amin (1986) and Moskalenko (1984) and were higher than those obtained by Reddy et al. (1984). These may probably due to the different breeds studied by them and due to the different plane of nutrition. The average birth weight (kg) of the different breeds recorded were found to vary significantly (P < 0.01) These were similar to the

findings of Mali et at. (1985) and Cho et al. (1988). The average birth weight (3.38 + 0.12) of Patanwadi lambs (native breed) was found to be significantly different from the lowest weight (2.45 + 0.16) recorded in 3 RP lambs. These could probably be attributed to the high level of exotic blood in 3 RP breeds.

The average birth weight did not vary significantly with regards to the sex of the lamb born.

Time of lambing : A total of 82 lambings were covered in this study involving 16 Patanwadi ewes, 22 MP ewes, 27 RP ewes, 10 5 RP ewes and 7 3 RP ewes. The maximum lambing occurred from 0.01 to 9.00 hrs. which accounted for 74.39 percent of the total lambings and from 12,01 to 21.00 hrs, which accounted for 21.95% of the total lambings. The lambing percentage remained lowest during 9 to 12 hrs. period both of morning as well as night. The maximum lambings in the individual breeds occurred between 0.01 to 3.00 hrs. in P. MP and RP: between 3.01 to 6.00 hrs. in 5 RP and between 6.01 to 9.00 hrs. in 3 RP. respectively, whereas the minimum lambings in these breeds occurred between 9.00 to 24.00 hrs. The findings were in agreement to those of Kaushish and Sahni (1975), but differed from those observed by Attoshi and Osterberg (1980) which could be due to different breeds studied and due to the different climatic conditions.

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Rate of twinning and sex ratio : Out of the 82 lambings recorded, only one incidence of twining in RP breed was observed. The incidence of twinning in RP was 3.70% (1 out of 27). Out of the total of 81 single lambings 41 male and 40 female lambs were born, giving a ratio of 50.62: 49.38 male to female lambs born.

## ACKNOWLEDGEMENT

The authors are thankful to the authorities of Gujarat Agricultural University for providing necessary facilities to conduct the study.

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## CHRONIC PYOMETRA IN A BITCH - A CASE STUDY

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Cystic endometrial hyperplastic and pyometra complex is one of the common condition encountered in bitches. In attempt to understand this complex disease, a detailed case study was conducted on a clinically affected bitch that was presented with purulent discharge at the gynaecology unit of veterinary college teaching hospital, Pondicherry.

**Case history :** The bitch was ten years old and nulliparous. It was reported that the bitch had oestrual bleeding 40 days back. The bitch was said to be having high temperature, anorexia, taking more amount of water and frequently vomiting without any vaginal discharge when it was first presented to this hospital. The detailed anamnesis revealed that the bitch had similar type of symptoms with purulent discharge from the vagina two year back.

**Clinical examination :** The animal was presented in lateral recumbency, dull, depressed, toxaemic and had rectal temperature of 100.4 F. The

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respiration was shallow and had weak pulse. Abdominal palpation revealed enlarged uterine horns. Light brown coloured haemorrhagic purulent discharge with fetid odour was noticed from the vaginal passage. The case was diagnosed as pyometra of open type and decided to perform emergency ovariohysterectomy. Before the operation, blood sample was collected for hematological and biochemical studies and uterine swabs were collected for culture and antibiogram. Under general anaesthesia, the surgery was performed on the right flank region as per the standard procedure. The removed uterus was enlarged and filled with two litres of haemorrhagic purulent exudates. The ovaries revealed presence of corporalutea in both the ovaries. Pieces from both the uterine horns were collected for histopathological studies. In spite of post-operative care the bitch died the next day.

## **RESULTS AND DISCUSSION**

### Haematological parameters:

R.B.C.	: 5.5 x 10/cmm
Haemoglobin	: 8.25 gm%
W.B.C	: 33,400

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<b>Differential Le</b>	eucocyte count:
Neutrophils	: 67%
	(47% band cells)
Lymphocytes	: 32%
Eosinophils	: 1%
<b>Biochemical</b> p	parameters:
Creatitine	: 8.98 mg%
B.U.N.	: 40.41 mg/dl
	and a balance of the

**Bacteriological studies :** In the antibiotic sensitivity tests cephalexin, gentamycin, cloxacillin, cotrimoxazole, tetracycline and erythromycin were found to be resistance and sensitive only to



Fig. 1. Hyperplastic endometrial glands with desquamation of endometrial epithelium

ciprofloxacin. *Escherichia coli* was isolated on culturing of the swab obtained from the uterine lumen.

Histopathology of the uterine horn : Histopathology of the endometrium (Fig. 1 & 2) showed marked degenerative and necrotic changes in the lining epithelium with desquamation and infiltration mononuclear cells consisting of plasma cells, lymphocytes and macrophages. Focal fibroplasia and sub-endometrial fibroplasias were also noted in certain regions. The endometrial glands revealed hyperplastic and cystic changes with

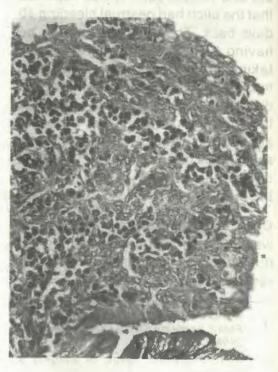


Fig. 2. Degenerative lining epithelium with mononuclear cells infiltration.

within the lumen and engorgement of the blood vessels in the myometrium.

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In this case the predisposing factors are the old age and nulliparous condition. This agrees with the opinion of Dow, (1958). The uterine discharge was having E.coli. This confirms with the reports of Sandholm et.al (1975), who reported that during luteal phase. the endometrium is having more affinity for E.coli organism. The present case was also in the progestational phase as both the ovaries had corpra lutea. There was elevation of the leucocyte count with lot of immature neutrophils. In bitches. bacterial infection with localization and

In some pases, J-Mile (1-2 teaspo

faintly eosinophilic cellular exudates pus formation stimulates a marked neutrophilia in comparison to a generalized infection. Infiltration of more macrophages indicates to macrophage monocyte transformation, probably due to chronic infection. The endotoxins released by the E.coli caused severe toxaemic condition of the bitch. There was very much increase in the B.U.N. and serum creatitine values suggestive of renal dysfunction which may be due to the endotoxins released and formation of immune complexes as reported by Sandholm et. al (1975). Histopathological finding in this case agree with the type IV of cystic hyperplastic complex in the dogs (Dow, 1958).

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# CLINICAL USE OF POLYETHYLENE POLYMER IN BOVINE OBSTETRICS

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Dystocia cases are treated as emergency and a careful examination is essential to establish the correct diagnosis and obstetrical manoeuvres to deliver the fetus pervaginum. In many cases examination is difficult. especially in a protracted dystocia, with swollen and dry mucous membranes of birth passage, live/ dead fetus, and a tightly contracted uterus making it impossible to carry out any obstetrical manoeuvers (Roberts, 1971). The present paper deals with the Clinical use of a commercial lubricant Polyethylene Polymer (J-lube) Product of Jorgensen Laboratories, Loveland, Colorado, USA in the management of dystocias in bovines.

### CLINICAL STUDY

Seven cross bred cows and two murrah graded buffaloes with the history of protracted dystocia (>36-48 hrs.) were included for the study. Both maternal (imperfect cervical dilatation and fetal (positional and postural abnormalities and fetel emphysema) factors were encountered as cause of dystocia. Under low caudal epidural anaesthesia (2% Lignocaine HCI-Xylocaine Astra - IDL) a thorough clinical examination revealed loss of fetal fluids, dry birth passage and fetus, rendering obstetrical manoeuvring difficult. Therefore it was decided to use polyethylene Polymer (J-Lube) as a intrauterine liquid replace following the method suggested by Carleton (1994).

Approximately 1/4 of a cup of J-Lube powder was placed in a bucket. The bucket was first filled half full with warm water and stirred well to get it into a solution. Then the bucket was filled with more warm water. The stringy solution thus prepared was introduced by gravity into the genital tract as far anteriorly as possible over the dorsal aspect of the fetus with a rubber tube attached to an enema can. in some cases, J-lube (1-2 teaspoons) taken in the palm was carried into the birth canal and was smeared directly over the surface of fetus and birth passage. After introducing J-lube both positional and postural abnormality of the fetus could be easily corrected and in certain cases partial fetotomy operations were performed in order to effect delivery of the fetus pervaginum. Repulsion and subsequent forced extraction procedures were found to

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be very easy. Pervaginum examination following relief of dystocia revealed no laceration/tear to the birth passage.

### DISCUSSION

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In difficult or prolonged dystocia, loss of fetal fluids renders obstetrical manipulation/ operation Intrauterine liquid difficult. replacement as lubricant is perhaps the most important procedure in preventing or minimizing trauma to birth canal during assisted delivery and also manipulations inside the birth canal like repulsion and traction of the fetus (Sloss and Duffy, 1980). In cases requiring large guantity of replacer and lubrication epidural anaesthesia is necessary to prevent the animal straining and throwing out the lubricant. This provides sufficient relaxation so that lubricant can flow about and cover the fetus and uterine. cervical, vaginal and vulvar walls. (Roberts 1971). The commonly used intrauterine liquid replacers are non irritant soapwater, mineral oils, mucus obtained from linseed and

methylcellulose powder in hot water, with suitable preservative (Sloss and Dufty, 1980). The above liquid replacer are to be prepared well in advance and kept ready for use during calving season. The commercial preparation J-lube (polyethylene Polymer 25%, dispersing agent 75%, Net wt. 284g) has the advantage of being nonirritant, nonstaining, colourless and odourless powder easy to prepare quickly at the time of handling dystocia. It is effective and safe for both the obstetrician and animal. The J-lube can be easily washed and removed from the obstetrician hands and from the floor by washing with water. It is non irritant and does not become rancid. From the above clinical trial it is evident that J-lube serves as a perfect lubricant in all prolonged cases of dystocia.

### ACKNOWLEDGEMENT

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## DYSTOCIA DUE TO HYDROCEPHALUS IN PANDHARPURI BUFFALO

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Hydrocephalus is dropsy of brain and denotes swelling of the cranium due to slow accumulation of excessive cerebrospinal fluid in and around the brain. It can arise whenever there is obstruction to the free passage of the cerebrospinal fluid into the arachnoid spaces. Death results due to pressure on vital centres in the brain (Roberts, 1971 : Sane et.al. 1994).

### CASE REPORT

A pluripara Pandharpuri buffalo aged about 9 years at full term pregnancy with a history of dystocia was admitted to the veterinary polyclinic. The buffalo was straining hard with the water bag presented at vulva. Per-vaginal examination and uterine exploration after lubrication revealed completely dilated cervix. The calf was dead, presented longitudinal and posteriorly in lumbosacral position with both the hind limbs extended in the dilated birth canal. The

2.Assistant Professor - Animal Reproduction, Gynaecology & Obstetrics.

3.Associate Professor - Veterinary Surgery and Radiology. head of the calf caused the problem and there was difficulty of delivering the head. The dystocia was relieved by subcutaneous embryotomy coupled with judicious forced extraction with ample lubrication.

A fully developed female calf with the distension of head due to fluid accumulation in the cranial cavity was delivered. The skin over the distended mass was intact with hairs on it. When the cranium was opened the frontal, temporal and parietal bones were deformed with a marked thinning of these cranial bones. The fluid in the cavity was colourless measuring about 3 litres. The other components of calf body were fully grown and did not show any abnormality.

Hydrocephauls when severe enough results in dystocia that cannot be relieved by mutation and forced extraction (Roberts, 1971). In posterior presentation intrauterine diagnosis of this condition is difficult, Cephalotomy is not possible and ceaserean section may be necessary in the female (Arthur, 1989).

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### HYDROALLANTOIS WITH CHARACTERISTIC PLACENTAL CHANGES IN A COW

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Hydroallantois, dropsy or hydrops of allantois is the single factor present in 85-90 percent of the dropsical conditions affecting the bovine foetus and its membranes (Roberts, 1971).

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A Jersey cow belonging to the University Dairy Farm, Palampur, had completed the gestation length. Inspite of straining and presence of water bag at vulva for the last 2-3 hours, the animal failed to complete second stage of labour. Externally, the abdomen appeared distended slightly more than the normal. On pervaginal examination, the exposed water bag was found to be thickened and tough, while cervix was fully open. The water bag was ruptured leading to gush of abnormally large volume of fluid. Thereafter, the amnion was ruptured and a live foetus with ascitis was delivered, but it died after one hour of birth. The foetal membranes were also removed and these exhibited characteristic raised areas between the allantochorion space (Figure). Post operative therepy included oxytocin, antibiotics and other

supportive therapy.

Hydroallantois of varying intensities in cows develops rapidly within 5-20 days during last trimester of pregnancy. The present case was of mild nature as it could be diagnosed at the time of parturition. Uterine inertia because of distension by large amount of allantoic fluid (Roberts, 1971) could be the reason for delayed completion of the second stage of labour in this case. The presence of accessory cotyledons on placenta in the present case, further confirms placental dysfunction and diseased uterus (Arthur et al., 1989)



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## UTERUS DIDELPHYS IN A HEIFER

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Developmental defects of the Mullerian ducts lead to various anomalies of the vagina, cervix and uterus. Failure of fusion of the median walls of the Mullerian ducts in the cervical region and in the region of uterine body leads on to a condition called uterus didelphys. In such cases each uterine horn connects with the vagina by a separate cervical canal (Arthur et al. 1985). In animals having true uterus didelphys with a double cervix, conception may be delayed if they are artificially inseminated because the semen may be deposited in the cervix on the side opposite to the ovary from which ovulation takes place. Roberts (1971) has observed several 'repeat breeder' cows with this type of uterus. Even if they conceive since only one horn takes part in the placentation of the foetus, abortion, premature birth, retained placenta and infertility are more common in these cows than in normal animals.

A three year old Jersey crossbred heifer with the history of more than a dozen inseminations from a local artificial insemination centre was presented for examination. Detailed clinico-gynaecological examination of the said animal revealed the existence of double cervix. On further examination of the genial tract after introducing one pripette each into both the crevial canals, the condition was diagnosed as uterus didelphys. Examination of the genitalia recovered after sacrificing the animal confirmed the condition as uterus didelphys (Fig.) F

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