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Scope and Limitations of Hormone Therapy in Animal Reproduction and Production*

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The goal of animal science research is to provide quality food to feed the growing need of human population. As the production directly depends on reproduction, optimum reproductive efficiency is a pre-requisite for animal production. The optimum reproductive efficiency and productivity can be achieved by (1) genetic of selection animals for high reproductive efficiency and production traits (2) ideal management conditions (3) proper feeding with due considerations for the physiological needs of the animals and the production. However, when these measures fall short in achieving the goals, a corrective hormone therapy is necessary.

The buffalo contributes to a major share of milk and meat production in the tropics. However, it is a difficult breeder because of its inherent susceptibility to environmental stress which causes subestrus and anestrus. These two conditions are responsible for a prolonged intercalving period resulting in great economic losses to the dairy industry. However, the studies of Roy et.al. (1968) have demonstrated that the buffaloes breed during summer if the heat stress is reduced. Jankiraman et.al.(1982) demonstrated that improved managemental practices could reduce the age at puberty in Surti buffaloes to 16-18 months and the intercalving period to 13-14 months.

When the ideal managemental practices fall to achieve the targets, hormone therapy is indicated. Singh et.al. (1979) have shown that 75% of subestrus buffaloes could be induced to estrus when treated with prostaglandins. Other drugs, such progestogens and PMSG (Pranee, 1985), clomiphene citrate (Dugwekar et.al., 1980)have also been found useful in the induction of estrus in anestrus animals.

Prolonged post-partum anestrus is another common problem, particularly amongst high milk producers and buffaloes. Although high plane of nutrition and positive energy balance helps in reducing the incidence of post-partum anestrus, the use of prostaglandins (Wichtel, 1991) and GnRH (Oxender, 1991) in dairy cows and prostaglandins in buffaloes (Singh, 1984) has been very effective in reducing the intercalving period.

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The above said methods of therapy have a limited value as they can be considered only for a few selected cases as corrective measures. Further, these treatments can only restore the physiologically normal rate of reproduction. However, the modern biotechnological procedures, such as superovulation, embryo transfer, embryo-splitting, cloning etc., can further exploit the reproductive capacity of food animal to an unimaginable magnitude.

The embryo transfer technique, first reported by Heape (1890) in mammals has the greatest potential for improvement. During the last few decades, the technique has been simplified so that it could be performed non-surgically. Further, the donors could now be superovulated to produce large number of embryos, the embryos could be frozen, could be bisected and the embryos could be cloned by mircromanipulation. The technique of in vitro fertilization, first developed by Chang (1959) in rabbits, is now being used in a large number of domestic animals and man, Currently, the procedures for the retrieval of oocytes from the cattle by trans-vaginal ultrasound guided probes are being developed which will enable more frequent oocyte retrieval even from a pregnant animal without disturbing the pregnancy.

The genetic engineering tools are also being successfully used to synthesize hormones such as recombinant bovine somatotropin which is capable of increasing the milk yield of well fed cows by 25 per cent. With these developments in the field of science and technology, the animal scientists hope to meet the challenge of "food for all " in the 21st Centrury.

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One step Freezing of immature Buffalo Oocytes

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ABSTRACT

Formation of ice crystal during freezing of cell is real problem and reduces the cell viability. Vitrification is an advanced technique of freezing in which the high concentration of cryoprotectant is used and result into the cell dehydration. Vitrification agent are classified into two group external and internal cryoprotective agent. The objective of the present study was the optimisation of the concentration of Ethylene glycol as vitrification agent. 4 M Ethylene glycol along with 0.25 M sucrose proves to be the best cryoprotective agent as it had the maximum maturation and fertilization role.

The possibility of harvesting oocytes from ovaries of highly productive soon after their slaughter and their in vitro maturation and fertilization to yield the genetically superior embryo has given the new hope for conservation and multiplication of high yielding buffaloes through embryo transfer technology. The oocytes collected from the slaughtered animal can be cryopreserved immediately after collection from ovaries and transferred to disired laboratory for in viro maturation and in vitro fertilization at convenient time.

Conventional methods of cryopreservation of oocytes demand the use of expensive equipments and are time consuming. Advanced freezing technique 'vitrification' is very quick and does not require expensive freezing machine. In this method the cells are suspended in high concentration of cryoprotectant at 5^{o}c are directly plunged into liquid Nitrogen. During vitrification water of the cells is directly transformed from liquid to glass like substance without formation of ice crystal which otherwise can cause the cell damage. The present study was taken to determine the concentration of ethylene glycol ideal for vitrification of buffaloes oocyte.

MATHERIAL AND METHOD

Buffalo ovaries were procured from abattoir (10-15) min) post slaughter. Ovaries were raised with warm (35-37^{o}c) Dulbeco phosphate Buffer saline (Gibco Laboratories, U.S.A) and transported in DPBS supplemented with penicillin (Hi Media, Bombay)4000 Units/lit in thermos flask.

After 5-6 washing in warm DPBS and one washing in 70% ethanol small to medium size (2-6 m.m) antral follicle were aspirated with 18 gauge needle attached to 2.0 ml syringe. After collection follicular fluid was poured into sterile petri plate (Torson, India) with grid on bottom outer surface. Oocytes were searched under stereozoom microscope (Baush and Lomb, U.S.A) Oocytes with

cumulus oophorus complex were picked up using unopette attached with mechanical aspirator and were given serial washing in 50 μ drop of T.I. hepes.

Four groups of vitrification mixture were prepared using 0.25 sucrose and varied concentration of Ethylene glycol (2M,3M,4M,5M) in DPBS and supplemented with 4% B.S.A. Fifth control groups did not contain Ethylene Glycol. The oocytes were picked up from TL Hepes washing medium and exposed to cryoprotective agent (5^{o}c) in gradually increasing concentration at o.5 M, for one minute each before placing them in final concentration of respective group. Ten oocytes in about 140 μl Vitrification mixture (5^{o}c) were drawn in 0.25 ml french straw under negative pressure and plunged into liquid nitrogen.

After 48 Hours vitrification the content of three straws of same group were expelled on a sterile petridish. The dilution and removal of cryoprotectant was done in the same order of that of its addition. Oocytes regained their normal contour and turgidity in the process. The oocytes were washed three time in P.B.S enriched with 10% fetal calf serum. (F.C.S. Sigma) and examined for morphology under stereozoom microscope. The good quality oocytes further processed for in vitro maturation followed by in vitro fertilisation adapting standard procedure.

RESULTS AND DISCUSSION

There was a significant difference inoocytes maturation rates between different treatment groups. The maturation rate of vitrified oocyte in Gr.I,II,III & IV were 18.18% 42.85% 73.07 % and 36.66% respectively. There was no oocytes maturation incontrol group.The oocytes after in vitro maturation were selected for in vitro fertilisation. As the oocytes of control group (v) did not matured, they were not placed for fertilisation. The fertilisation rates were 4.54% 19.52% 34.61% and 23.33% respectively.

Nag (1995) reported 42.07% maturation rate in immature buffalo oocytes vitrified in the medium containing ethylene glycol (3M) & sucrose (0.25M) which was higher than the maturation rate observed with DMSO and sucrose combination. The maturation rate in present study with 4M ethylene glycol and 0.25M sucrose was higher than that reported by Nag (1995).

Arav et.al (1993) vitrified the oocytes using a combination of sucrose with DMSO & propylene as cryoprotectant and they reported highest (80%) maturation rate with oocytes vitrified with 40% propylene glycol and 0.25M trehalsoe. In the present study the oocytes exposed to designated concentration of cryprotectant with one min exposure at 0.5 M rise in concentration. Shaw and Bernold (1992) used the same procedure for vitrification of mouse oocytes, but they kept the exporuse period as 15 seconds. They found the maturation rate77.80%. This indicate that the exposure period is also a critical factor in the effectiveness of cryopreservation.

The oocytes after invitro maturation were subjected to in vitro fertilisation with fresh capicitated buffalo spermetozoa. Nag (1995) vitrified immature oocytes using three combination of cryoprotectant containing

a critical factor in the effectiveness of cryopreservation.

The oocytes after invitro maturation were subjected to in vitro fertilisation with fresh capicitated buffalo spermetozoa. Nag (1995) vitrified immature oocytes using three combination of cryoprotectant containing DMSO. EG., propylene glycol with sucrose (0.25%) & reported fertilisation rate 15.23 % The fertilisation rate observed in this study Gr.III was 34.61% indicating that 4M ethylene glycol and 0.25 sucrose showing the best result.

Lee and Chen (1991) and Hochi et.al (1994) have also reported ethylene glycol as superior cryoprotective agent for cryopreservation of immature buffalo oocytes. However, Hunter et.al 91995) reported a fertilisation rate of 42.9% for G.V stage bovine oocytes with 2m DMSO & 17.8% with 2m propylene glycol.

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Cryo-Preservation of Caprine Oocytes by Vitrification

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ABSTRACT

Sensitivity of caprine oocytes (fresh and mature) to cryopreservation by vitrification has been examined. Exposure of oocytes to the vitrifiation solution and very rapid cooling caused a slight loss in maturation ability.

Classical method of oocyte/embryo preservation requires expensive controlled rate biological freezer. Preservation of embryos by vitrification has become a new promising approach in the field of cryobiology (Rall and Fahy, 1985 Massip et al 1987 Agrawal and Polge 1989 Agrawal et al. 1994) Information on vitrification of oocytes of mammals and of goats in particular is meagre. In the present experiment post-vetrified changes in fresh and mature caprine oocytes have been studied.

MATERIAL AND METHODS

Caprine ovaries of abattoir origin were used as a source of oocytes. Immature oocytes were recovered by puncturing the follicles will the help of a 18 G hypodermic needle. Only the oocytes enclosed in compact multi layer cumulas cells and with evenly granulated cytoplasm were used.

Vitrification Procedure The Vitrification procedure used by Agrawal and Polge (1989) for mouse embryos with minor modifications was used. Good quality oocytes after 2-3 washings in Dulbecco's PBS oestrous goat serum were exposed to intracellular cryoprotecting medium (10 %) glycerol, 20% 1-2 propane diol in PBS) for 10 minutes. Embryos were then transferred to extracellular vitirification medium (25 % glycerol 25%) 1-2 propane diol in PBS The temperature of cryo-protecting and vitirification media during embryo exposure was maintained at $4^{o}C$ in an ice bucket 5-6 oocytes in vitrifiation medium and diluent (1M sucrose) in determined proportions were loaded in 0.25 ml straw. The straw was plunged in LN_2 in 2 steps. (i.e the portion of the straw up to the level of oocytes in vitrification medium plunged immediately and then rest of the portion progessively). After storage in LN_2 for different periods, the straws were taken out and thawed in water both maintained at $20^{o}c$ contents of the straw were mixed by shaking, emptied in a petri dish and left for 10 min at $4^{o}C$ maintained in an ice bucket, Sucrose was gradually diluted

in 0.5M and 0.25M sucrose in PBS After 3 washings in PBS oocytes were examined for morphological damages and nuclear configuration.

In Vitro Culture of Oocytes. Immature oocytes (both fresh and virified) were incubated in CO_2 incubator maintained at 38°c temp. 95 % humidity and 5% CO_2 in air. TCM 199 enriched with 20% goat serum, D-glucose and sodium pyruvate was used as culture medium, After 24 hrs incubation, oocytes were fixed and stained with aceto-orcein to observe nuclear changes.

RESULTS AND DISCUSSION

Three hundred and twenty seven oocytes graded as good were used for vitirifcation. Similarly 414 mature oocytes were used for vitirification. Number of oocytes last during processing before plunging in liquid nitrozen, oocytes recovered after thawing and their morphological and nuclear status are presented in Table.1

Table.1 Effect of Vitrification on fresh and mature oocytes

Morphological and nuclear changes	Fresh oocytes	Mature oocytes
1. No. of Oocytes used for vitrification (No of replicates	327 (21)	414 (39)
2. No of Oocytes lost/damaged before plunging in LN2	3	1
3. No of oocytes vitrified	324	413
4. No of Oocytes thawed	324	229
5. No of Oocytes recovered	304	206
6. No. of recovered oocytes exhibited morphological changes	32	26
7. No of recovered oocytes examined for nuclear changes	-	-
a. Germinal Vesicle (GV)	72	3
b. Germinal Vesicle Break Down (GV BD)	36	1
c. Metaphase I (Mer-I)	57	13
d. Anaphase I (Ana-I)	1	1
e. Telophase I (Telo-I)	-	-
f. Metaphase – II(Met-II)	-	37
g. Oocytes without chromatin material	39	36
h. Condensed	23	37
i. Total	228	128

In vitro Maturation of Vitrified Oocytes: To check the abilityof vitrified oocytes to mature and fertilize in-vitro, one hundred ninteen oocytes were thawed using 20°C water bath and after step-wise dilutionof sucrose. The Oocytes were kept In culture in TCM - 199 supplemented with 20% oestrous goat serum in CO_2 incubator at 38°C temp. 95% R.H. and 5% CO_2 in air. After 24h of culture the oocytes were fixed and stained with acetoorcein to observe maturation change (table.2)

Table.2 In-Vitro Maturation of Vitrified Oocytes

1.	No of vitrified Oocytes cultured	119
2.	No of Oocytes studied for nuclear changes after culture	61
3.	Stages of meiosis	-
a.	Germinal vesicle	1
b.	Germinal Vesicle Break Down (GVBD)	-
c.	Metaphase – I (Met-I)	1
d.	Anaphase – I (Ana-I)	-
e.	Telephase (Tele-D)	-
f.	Metaphase (Met-II)	20
g.	Oocytes without chromatin material	29
h.	Condensed	1

In-Vitro maturation of vitrified Oocytes stored in LN_2 for more than a year: One hundred forty vitrified Oocyes after storage in LN_2 for more than 1 year were thawed in a water bath maintained at 20°C Thawed oocytes were examined for morphological changes. Recovery rate was 92.8% (130/140) One of 130 Oocytes recovered 10 Oocytes exhibited damage in zona pellucida and ooplasm. 120 oocytes were cultured for 24 hours in CO_2 incubator under similar condition as above, They (25%) oocytes reached to metaphase II stage. The study reveals no adverse effect on oocyte maturation after long storage.

In the present study 33 and 25 per cent oocytes matured to metaphase. If stage after storage in LN_2 for 1-4 months and 18 months respectively. Both values are significantly lower from that of in-vitro maturation rate of fresh oocytes reported earlier from our laboratory (Agrawal, 1992, Agrawal et.al 1995) Exposures of oocytes to the vitrification solution and very rapid cooling caused a slight loss In maturation ability.

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Augmentation of post-partum reproductive efficiency by the use of GnRH in buffaloes

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ABSTRACT

Twenty four buffaloes 30 ± 2 d postpartum were used to study the efficacy of GnRH (Receptal) treatment for an early induction of estrus. The results indicated that 12 ug of GnRH injection after 28 d parturition had beneficial effect in resuming ovarian activity and estrus significantly earlier thereby increasing conception rate. Further role of blood glucose level on reproduction have been implicated.

The factors like, summer stress, inherited silent subestrus, seasonality and long postpartum estrus intervals jeopardize the farmers' economy by increasing the calving interval and thereby reducing the calf crop and milk production in buffaloes.For resumption of ovarian activity and ovulation a highly potent synthetic analogue of GnRH (Buserlin) have been used in cattle (Britt et. al 1974; Cavestany & Poote, 1985; Hussein et. al 1992)

However, very scanty literature is available on its use in buffaloes (Aboul-Ela et.al 1985). The study under report was undertaken to determine the efficacy and economic use of GnRH (Receptal) for early induction of fertile estrus in post parturient Buffaloes to improve their reproductive performance.

MATERIAL AND METHODS

Twenty four post parturient buffaloes maintained at the Punjab Agricultural University Dairy Farm were used for this study. The animals were kept under loose housing system. They were provided with normal ration consisting of seasonal green forages and chopped wheat straw ad.lib_Concentrate mixture was provided as per their requirement. They had free access to fresh water;

Animals were randomly allocated to three groups. Group 1, was untreated as control; group 2 and 3, received intravenously either 8 or 12 ug GnRH, a synthetic analogue (Receptal, Hoechst, Bombay) respectively, on 30 ± 2 d post partum. Blood samples were collected from all the animals until day 90 postpartum to monitor their general health status.

The ovaries and uterus were examined per rectum twice weekly to study the involution of uterus and resumption of overian activity. Detection of estrus was done twice daily by parading vasectomized bulls. Artificial

insemination were done in buffaloes found in estrus with frozen thawed semen in mid to end of estrus.

Haemoglobin, packed cell volume and blood glucose were estimated as per standard methods The data on reproductive efficiency in term of ovarian activity, Involution of uterus, 1st postpartum estrus, conception rate etc., were recorded and subjected to statistical analysis described by Snedecor & Chochran (1967).

RESULTS AND DISCUSSIONS

Uterine involution was completed in all the buffaloes by d 31 which in confirmity with our earlier findings (Chauhan et al. 1977).

The data on reproductive and blood parameters have been presented in the table. Data revealed that the ovarian activity resumed significantly earlier in buffaloes treated with GnRH especially those receiving 12 ug (24 d) as compared to untreated ones (32 d) The results indicated that use of higher dose 12 (ug) seemed to be more effective than the lower dose 8 μg.

The finding that GnRH treatment enhanced the occurance of ovarian activity is in accordance to other reports in dairy cattle and buffalo (Schams et.al, Britt et al 1974 Aboul-Ela et al. 1985).

Like ovarian activity, onset of postpartum estrus was the longest in the control group. Both the doses of GnRH were found to decrease the days taken for the onset of postpartum estrus possibly by altering the endocrine milieu and/or reducing the incidence of silent heat. Our findings support the earlier findings both in buffaloes Aboul-Ela et al (1985) and dairy cattle (Fonsecs et al, 1980).

The data revealed that the service period varied from 115 to 123 days, This is in confirmity with our earlier reports Singh et al. However, 12 ug of GnRH has reduced the service period marginally. Data further revealed that GnRH treated group which had better blood glucose had better reproductive performance. Whether the performance was better due to higher glucose level or higher dose remain to be ascertained. It has been observed earlier that the buffaloes having higher blood glucose performed better (Sharma et al. 1996) and the number of service per conception has been considerably reduced from 2.1 to 1.4 and percent conception rate was also increased in the treated group. However, the value of haemoglobin and packed cell volume remained similar in all the groups indicating that the animals had good health and nutritional status ((Payne et al 1973).

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Table.1 Mean values of reproductive and blood traits for postpartum buffaloes treated with GnRH

	Control (6)	Group I (8)	Group II (5)
	Days		
Ovarian Activity	32.4 ± 3	27. 3 ± 4	* 24.0 ± 2
Postpartum estrus	44.3	39.5	* 37.0
Service period	119.3	123	114.8
Services per conception	2.1	1.9	*1.4
Conception rate (%)	38	38	*50
Blood glucose (mg %)	47.5	47.3	*57.4
Haemoglobin (g %)	11.9	12.1	12.3
Packed dell volume (%)	22.1	22.4	21.9

*Significant ($P \leq 0.05$) Figures in parenthesis indicate the number of animals.

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Estrus Synchronisation And Fertility with the uterine infusion Of Prostaglandin F_2 alpha and Lugols Iodine in cross Bred cows

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ABSTRACT

The Comparative efficacy of two treatments viz., prostaglandin F_2 alpha and Lugels iodine were evaluated for induction of estrus and restoration of fertility in subestrus cows. Induction and synchronisation of estrus was easily achieved by the prostaglandin F_2 alpha. On the contrary intre-ulterine infusion of lugel's idine resulted in higher expression of estrus but the synachronisation of estrus was not precise. Ovulation and conception rate achieved with each treatment was comparable to control.

In order to reduce the dose and consequently the cost of prostaglandin F_2 alpha for induction of estrus through intrauterine route has been tried by various workers (Chauhan et al. (1989). Certain irritant solution such as Lugol's iodine (1:2) has also been tried for induction of estrus by Nakahara et.al (1971) and Ginther and Moley. (1972). But the comparative efficacy of these two drugs for inducting/synchronization of estrus in cows are very scanty. Therefore, the investigations was carried out in above perspective.

MATERIALS AND METHODS

The present research was conducted at Livestock Farm Adhartal. Jabalpur during the period. 89-90 Animals not exhibiting estrus (90-250 days) post-partum. .And having a palpable corpus luteum in one of the every without apparent affections of the reproductive tract were randomly divided into three groups;

Group-1 (#17) Prostagiandin F_2 alpha (5ng) was given intra ulterine.

Group-II (#21) Lngel's iodine (1:10) 20ml intra ulterine (Herman and Kadden, 1953.

Group-III (# 14) intreated serve as control received 20ml normal saline solution. Intra uterine

The detection of estrus was done with the help of teaser bulls twice a day. In addition rectal palpation was done on alternate day up to a period of 21st day post treatment. The animals detected in estrus were inseminated with liquid semen extended in EYC dilutor twice a day at 12 hr apart. Ovulation was confirmed by detecting corpus luteum 12th day after induced entrus. The conception rate was noted by performing pregnancy diagnosis at 60 day post insemination

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Statistical calculation were done as per the methods of Snedecor and Cochran, (1968).

RESULTS AND DISCUSSION

The comparative efficacy of Prostaglandin F_2 alpha and Lugels iodine for induction of estrus and fertility response are presented in Table

Perusal of the Table 1 revealed that estrus was induced significantly earlier in the animals of group 1 (3.88 ± 0.57 days). This ranged quite nearer to the findings of Chauhan et.al. (1982) and Thakar et.al. (1989)

Synchronization of estrus was very precise in the animals of group I while it was not precise for the animals in other groups. This indicated that synchronization of estrus was better achieved by prostaglandin F_2alpha due to its luteolytic effect. Similar findingshave been reported earlier by Nackshara Et.al 1971.

The overt estrus symptoms were maximum in group 2 (Lugel's solution) Than in group 1 and control. This indicated that Lugel's solution treatment was better for exhibition of covert estrus then prostaglandin F_2alpha. This may be attributed to irritant property of Lugol's iodine or its better absorption through uterine mucosa.

The ovulation and conception rate was higher in group I (58.82%) that the other groups. This findings indicated that prostaglandin F_2 alpha given in lower doses (5mg) intra uterine is effective in estrus induction and restoration of fertility. Our findings support the findings of Ferara and Kumartillaka (1977) and Pathiraja et.al (1979)

Table.1 Showing response of subestrue cows within 21 days post-treatment

Groups	Interval between inj. to Occurrence heat (days)	No.of animals detected in estrus by teaser	Estrus detected By rectal Palpation	Prog
Group-I	3.88 ± 0.57	4	13	10 (50.82)
Group-II	7.00 ± 1.06	23	6	14 (48.27)
Group-III	11.71±2.56	10	4	4 (40)

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Quantitative and Qualitative changes in total proteins. Immunoglobulins and Cholesterol in Blood plasma of Buffaloes superovulated with different PMSG protocols

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ABSTRACT

Eight healthy murrah buffaloes was superovulated on day 12 of estrous cycle. All the animals were divided randomly into three groups prior to commencement of superovulation treatment. Each animal of group I (n=2) was given 2500 IU of PMSG $PGF_2\alpha$ on day 12 followed by an injection of 25mg on day 14. Animals of group II (n=3) were treated as in group I but additional injection of Anti PMSG (5 ml, 1:4) raised in rabbit was given 20 hrs after the onset of estrus. Buffaloes in group III (n=3) were given the same treatment as in group II but in addition PRID was inserted in the vagina of each animal on day 4 and removed on day14. The buffaloes superovulated with PMSG alone and PRID and PMSG+ANTI PMSG, showed a decrease in the immunoglobulin content. Higher superovulatory response was observed with PMSG (CL, 4.25 ± 2.9) and PRID+PMSG+Anti PMSG (5.0 ± 2.1) than with PMSG+Anti PMSG (4.0 ± 0.0) Embryo recovery was also higher in the former groups compared to the later. A decrease in the cholesterol content of blood was noticed in all the groups. Blood plasma protein level did not show any significant changes during superovulation in different treatment groups. PAGE pattern of partially purified immunoglobulins showed several alterations in the protein pattern of blood plasma which reflects varied metabolic adjustments taking place during superovulation treatment.

Low fertilization rates or complete absence of fertilization are the major limiting factors in superovulation procedures for embryo transfer in cattle and other farm animal species. Some of the losses can be attributed to endocrine abnormalities or some other metabolic and biosynthetic deficiencies associated with supervulation (Armstrong, 1993). Therefore the present study was planned to examine the relationship of blood plasma, protein, immunoglobulins and cholesterol levels with superovulation response and embryo recovery in buffaloes.

MATERIAL AND METHODS

Eight clinically healthy, normally cycling buffaloes between second to seventh lactation being maintained at the Punjab Agricultural University were used for this study. All the animals were divided randomly into three groups prior to commencement of superovulation treatment. Each animal in group 1 (n=2) was given 2500 IU of pregnant mare serum gonadotrophin (PMSG) on day 12 followed by an injection of Anti PMSG (5 ml, 1:4) raised in rabbit was given 20 h after the onset to estrus. Buffaloes in group III (n=3) were given the same treatment as in group II but in addition progesterone releasing intravaginal

device (PRID; Sonafi Animal Health Ltd.,) was inserted in the vagina of each animal on day 4 and removed on day 14 of the cycle prior to administration of prostaglandin F2 alpha. Blood was collected from all the animals by puncture of Jugular vein on day 0 (preceding superovulation treatment), 12, day of estrus and day of embryo collection. Blood plasma was immediately separated by centrifuging at 6000 rpm for 20 minutes. The plasma was analyzed for total protein content (Lowry et al., 1951). Immunoglobulins (Oser, 1965) and cholesterol (Ferrand and Roeffel, 1958). Immunoglobulins were characterized by polyacrylamide gel electrophoresis (Davis, 1964). The data is expressed as mean ± SD (Singh et al., 1984).

All the animals detected in estrus were inseminated 6,12 and 24 hr after the onset of standing estrus with good quality semen. Embryos were collected non-surgically on day 5 with German Catheter using Delbecco's phosphate buffered saline fortified with 0.4% bovine serum albumin and antibiotics at the standard rates.

RESULTS AND DISUCSSION

The buffaloes superovulated with PMSG, PMSG+Anti-PMSG and PRID+PMSG+Anti PMSG did not show any significant difference in plasma protein concentration from day zero to day of embryo collection.

The buffaloes superovulated with PMSG and PRID + PMSG +Anti PMSG showed an increase in plasma immunoglobulin content (Table.) in the blood plasma while it was decreased in the case of PMSG+Anti PMSG treated buffaloes. Superovulatory response and embryo recovery was also higher in the former treatments (Table.) However, In cattle, Goto et al., (1988), observed that the blood plasma concentration of gamma globulin was significantly higher in those animals that did not respond to superovulation than those yielding normal embryos after superovulation.

A decrease in the blood plasma cholesterol content was found in all the animals treated with the three superovulation treatments. It ranged from 3.40 g % to 3.57 g % on the day of embryo collection. The ovulation rate and embryos recovered varied between 4.0 ±0 to 5.0 ± 2.1 and 0.75 ± 0.35 to 1.75 ± 0.5 respectively (Table) Callesen et al., (1990) reported that in heifers with preovulatory estradiol surge, number of corpora lutea, follicles and transferable embryos were greater than those without and estradiol surge. The decreased cholesterol content from day zero to day of estrus/day of embryo collection in the present studies perhaps corroborates the observations of Callesen et.al (1990). Cholesterol is the precursor of progesterone, estradiol and estradiol surge occurs at the time of estrus. Cholesterol might be utilized for the synthesis of estradiol during the superovulatory process and thus the decreasing levels of cholesterol during the pre-ovulatory period can perhaps be used as an indication of a good superovulatory response.

Characterization of immunogbolulins : Eight to nine proteins were detected in the partially purified immunoglobuline of

blood plasma of buffaloes treated with PMSG, PMSG±Anti PMSG and PRID +PMSG +Anti PMSG

PMSG Treatment : In this treatment, the proteins with mol and wt. Of 15-175 KDa and 40-85 Kda were found in the blood plasma on day 12 and day of embryo collection, respectively, The proteins with mol of 90-120 KDa and < 12.5 KDa appeared on the day of estrus and showed an increase in their proportion up to day of embryo collection. A high mol.wt. protein of > 240 KDa showed an increase up to day of estrus with a sudden decline on day of embryo collection. Reverse was the case with 165-195 KDa protein which showed a sharp decline in its proportion on day of estrus. A<12.5 KDa protein of 0.47-0.57 relative mobility showed a sudden decline on the day of embryo collection.

PMSG+Anti PMSG Treatment Three proteins in this treatment with mol.wts. of > 240 KDa, 30-40 KDa and <12.5KDa showed a decrease in their proportion up to day of estrus and then disappeared on the day of embryo collection. A protein of 90-120 KDa appeared on the day of embryo collection .The proteins with mil.wts. of >240 KDa and 165-195 Kda, respectively showed a sharp decrease followed by an increase in their proportion on the day of estrus. The proteins of 220-240 KDa, 150-175 KDa 40-85 KDa showed a progressive increase and < 12.5 KDa of 0.47-0.57 relative mobility showed a progressive decrease in their proportion up to day of embryo collection.

PRID+PMSG_Anti PMSG Treatment In this treatment the proteins with mol wts. Of > 240 KDa and 90-120 KDa showed a sharp increase in their proportion on the day of estrus and 150-175 KDa and 40-85 KDa protein showed an increase in their proportion on the day of embryo collection. A 220-240 KDa protein suddenly declined on day 12 of the cycle. The proteins with mol.wt.>240 KDa of relative mobility 0.05 and 165-195 KDa showed their presence on day 0 and day 12 of the cycle. Three < 12.5 KDa proteins of relative mobilitiers 0.47-0.57, 0.58-0.65 and 0.70-0.75 showed a progressive decline in their proportion,

The alterations induced in the protein immunoglobulin pattern in the blood plasma with different superovulation treatments reveal that varied metabolic adjustments take place during superovulation. Since with PRID+PMSG+Anti PMSG, the best superovulatory response is achieved and about six of the proteins decreased in this treatment. It perhaps reflects the higher metabolic activity of increased utilization of certain proteins as Well as synthesis of some new proteins taking place in these animals to cope up with the increased ovarian activity during superovulation.

Table Protein, Ig and cholesterol contents (g %) in the blood plasma of Buffaloes in relation to supervulatory response and embryo recovery

Day of Superovulation	Hormonal treatment								
	PMSG			PMSG+Anti PMSG			PRID+PMSG+AntiPMSG		
	Protein	Ig	Choles Terol	Protein	Ig	Choles terol	Protein	Ig	Cholesl terol
Control	8.25 ± 1.18	3.07 ±0.46	4.57 ±1.40	7.95 ±1.06	3.97 ±0.35	4.42 ±1.17	6.50 ±0.27	3.65 ±0.89	4.60 ±0.92
D-O	7.90 ± 0.98	3.50 ± 0.67	3.80 ±1.15	8.20 ±0.99	3.65 ±0.43	3.55 ±1.12	6.65 ±0.52	4.22 ±0.54	3.67 ±1.65
D-8	7.25 ±0.74	2.92 ±0.49	3.65 ±1.25	8.05 ±0.78	3.30 ±0.32	3.50 ±1.08	6.90 ±0.34	4.0 ±0.47	3.75 ±1.05
D12	7.90 ±1.18	3.80 ±0.53	3.92 ±1.26	7.10 ±0.93	3.90 ±0.39	4.02 ± 0.57	7.30 ± 0.69	4.60 ± 0.93	4.37 ±1.40
DOE	8.75 ±1.69	3.65 ±0.68	3.67 ±1.70	7.20 ±1.09	3.32 ±0.50	3.92 ±0.89	6.75 ±0.68	4.60 ± 0.73	1.82 ±4.80
DOEC	7.75 ±0.72	4.07 ±1.23	3.57 ±1.44	8.30 ±0.93	3.50 ±1.20	3.40 ±0.39	6.90 ±1.16	4.60 ±0.55	3.47 ± 0.83
Superovulation Response	CL 4.25 ± 2.90 Embryos 1.25 ± 0.95			4.0 ± 0 0.75 ± 0.35			5.0 ± 2.1 1.75 ± 0.5		

D=Days, DOE=Day of estrus; DOEC=Day of embryo collection

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Effect of hcg on Serum calcium, Inorganic phosphorus and alkaline Phosphatase level during oestrous cycle in goat

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ABSTRACT

The levels of serum calcium, inorganic phosphorus and alkaline phosphatase were estimated during different stages of oestrous cycle in local goats of Assam, treated with different doses of hcg. Calcium and inorganic phosphorus did not differ significantly during different stages of oestrous cycle as well as amongst different experimental groups, whereas alkaline phosphatase levels showed significant difference (P<0.00) during different stages of oostrous cycle but did not differ amongst groups. Serum calcium and alkaline phosphatase levels were lowest on the day of oostrus (day 0) and then gradually increased to the peak values on day 12 of the cycle, followed by a declining levels on the subsequent oestrus (day O_1). Serum inorganic phosphorus levels did not show significant fluctuation during oestrus cycle.

The importance of superovulation, in recent years, has increased with the introduction of many exogenous superovulatory hermones either in combination or alone. Dietary mineral elements are known to affect the physiological function in general and reproduction in particular (Hidiroglou, 1979). Administration of any exogenous hormone may bring about certain changes in the serum mineral level as well as in the body tissues which is reflected through enzymic activity. Although, much works have been done on the role of mineral and enzymic activity in goat reproduction, there is a paucity of information regarding their serum levels specially after treatment with superovulatory hormones. Therefore, the present investigation was carried out to study the levels of serum calcium, phosphorus and alkaline phosphatase (AKP) in local goats of Assm at different stages of oostrous cycle after superovulating with hCG

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MATERIALS AND METHODS

A total of 40 sexually matured healthy local female goats of Assam, 2-3 years of age, were included in this study. The animals were maintained in semi-intensive system of rearing. The animals were divided into four groups viz., A, B. C and D, having ten animals in each group. The animals of group B, C and D received an intramuscular injection of 550 IU, 650 IU and 760 IU of hCG

(Cherulon*) at six hours post onset of oestrus, respectively; while animals of group A served as control and received an intramuscular injection of distilled water at the same time schedule.

From each animal, of all the groups, about 2-3 ml of blood sample was collected by jugular venupuncture at four days interval, starting from the day of oestrus (day O) to day 16 of the cycle and the last collection was done on the day of next oestrus (day O_1)Serum was separated and subjected to the analysis of calcium, inorganic phosphorus and alkaline phosphatase as per the methods of Webstar (1952), Taussky and Shorr (1953) and Wootton (1964), respectively. Data were analysed as per standard methods of Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

In all the groups mean serum calcium concentrations mg/100 ml) were lowest on the day of oestrus (9.05 ± 25 to 0 10.00 ± 0.17) increased gradually to highest levels on day 12 (10.20 ± 0.18 to 10.60 ± 0.32 of the cycle and subsequently declined on the day of next oostrus (9.27± 0.14 to 9.87 ± 9.19). The analysis of variance revealed no significant difference in relation to serum calcium during different stages of oestrus cycle as well as amongst the different groups. These findings are in agreement with earlier reports (Schultz et al ., 1971 and Nigam et al.,1990)> Low calcium concentrations observed on the day of oostrus might be due to the high levels of estrogen (Soliman et. al., 1964 and Bhattacharrya et al., 1975) During oostrus increase irritability and contraction of uterine tissue may occur due to low serum calcium levels, Moreover, calcium has been found to sensitize the female tubular genitalia for the action of steroid hormones (Moddie, 1965) Hence higher levels of serum calcium at different stages of luteal phase could be attributed to the demand for uterine tissue.

No significant difference was observed in the level of serum inorganic phosphorus amongst the different stages of oestrus cycle as well as different groups. However, the inorganic phosphorus levels showed apparently higher values during oestrus (8.21 ± 0.31 to 9.53 ± 0.18 mg/100 ml) thereafter the values showed fluctuations among different stages of oestrous cycle in all the groups; which reflected a positive association with its demand and utilization during oestrus cycle in goat (Nigam et.al., 1990 and Bhattacharrya et.al., 1995)

Although the serum alkaline phosphatase (AKP) levels showed no significant different experimental groups, a significant ($P<0.05$) difference was observed during the different stages of oestrus cycle. The levels of AKP(K.A units/100ml) in all the groups were lowest on the day of oestrus (9.18 ± 0.22 to 9.98 ± 0.24). Then the AKP levels continued to increase upto day 12 of the cycle (14.74 ± 0.10 to 15.04 ± 0.18) and thereafter, it declined till the day of subsequent oestrus (9.18 ±0.22 to 9.70 ±0.09). This trend of AKP level is in close agreement with that indicated by

*Chorulon – Chorionic gonadotrophin B. Vet.C. (Luteinizing hormone) Intervet, Holland.

Skjerven (1956) and Schultz et.al., (1971)In cows.Though AKP levels did not differ significantly amongst different groups, however, slightly higher peak value of 15.04± 0.18 K.A U/100 ml of serum AKP in Group C, as observed in the present study is quite in agreement with the findings of Jindal and Ratan (1992) in buffalo cows during diestrus phase after synchronizing the oestrus with $PGF_2 \propto$ and Smith et. al., (1970) in woman undergoing treatment with progestational agents.

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Superovulatory response and Embryo recovery in crossbred cows under field conditions

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ABSTRACT

Six gynaecologically normal crossbred Jersey Cows selected in different villages were superovulated with FSH-p and embryoes were flushed trans-cervically from the uterine horn on seventh day post-breeding. The superovulatory response, embryo recovery rate and quality of recovered embryoes were recorded. The mean number of corpus leutea (CL) and unovulatory follicles were 9.33 ± 0.17 respectively. The mean number of embryoes recovered from the left & right uterine horns were 2.83 ± 0.55 and 3.50 ± 0.70 with overall recovery rate of 71.43 percent. The overal mean number of total embryoes and transferabie embryoes recovered were 6.67 ± 0.79 and 4.33 ± 0.69 respectively.

Superovulation is a key element of embryo transfer programme in cattle. The high proportion of non-transferable embryos, resulting from both fertilization failure and degeneration of embryoes in donars' reproductive tracts before collection continue to be major limiting factors in current methods of superovulation (Armstrong.1993)

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The present investigation was designed to study the superovulatory response following FSH treatment in six cattle maintained at different villages.

MATERIALS AND METHODS

Six gynaecologically normal crossbred Jersy cows from different villages were selected. Double instramuscular injections of 25 mg PGF_2 ∝ (Lutalyse, Unichem, Bombay) at 11 days interval were given to each cow for synchronization of oestrum. All the cows were superovulated with 400 mg NIH of FSH-p (Folltropin –v, Vetrepharm, Inc., Canada) intramuscularly from day 10 of the oestrus cycle twice daily for four consecutive days at decreasing dose levels (66,54, 40 & 36 mgNIH units). Luteolysis was induced by PGF_2 ∝ given at 60 and 72 hours after the commencement of Superovulation treatment. Donars were inseminated with frozen semen at 24,36 and 48 hours following last PG injection. Superovulatory response was assessed per rectally by counting the number of corpora lutea and unovulated follicies in the ovaries. Non-surgical transcervical embryo recovery was done on seventh day after first insemination using modified Dulbacco's phosphate buffered saline supplemented with bovine serum

albumin (Elsden et al., 1976). The flushed out fluid from each uterine horn were evaluated using stereozoom.The mean number of ovulations between left and right ovaries were compard using 't' – tests (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

The mean number of C.L and unovulated follicle were 9.33 ± 0.72 (13 to 5) and 1.50 ± 0.17 (0 to 3) respectively. The total number of CL in the right ovary (31) is significantly ($P<0.01$) higher than left ovary (25). This agrees with the findings of Kharche et al., (1995) who recorded high ovulation in the right ovary than that of left ovary following superovulation. A significant different ($P< 0.01$) between the mean number of embryoes recovered from the left (2.83 ± 0.55) and right uterine horn (3.50 ± 0.70) was observed. The overall mean embryo recovered per cow was 6.67 ± 0.79 (ranged from 2 to 11) with recovery rate of 71.43 % The mean number of unfertilized ova, degenerated embryo and transferable embryoes recovered were 1.17 ± 0.41, 1.50 ± 0.42 and 4.33 ± 0.69 respectively. The overall fertilization rate was 82.50 % The low fertilization rate in the superovulated animals may be attributed to asynchrony between maturational events in the oocyte and follicle (Loos et al., 1991) including time of ovulation (Callesen et al., 1987). The percentage of transferable embryoes to the total number of embryoes recovered was 60. This agrees with the findings of Agarwal et al (1993), who reported that recovery of transferable embryoes varies from 45 to 75 percent.

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Follicular Growth, Atresia And ovulation Pattern in Guinea pigs treated with Different Doses of buffalo Follicular Fluid in Luteal Phase of the Estrous Cycle

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ABSTRACT

Guinea pigs were injected twice daily for 3 days with 0.1, 0.2 or 0.4 ml of buffalo follicular fluid (buff) treated with activated charcoal. The meantime to the onset of estrus was increased in a dose-related manner. The mean ovulation rates were not changed. However, 50% animals receiving 0.04 ml buFF twice daily failed to ovulate, Histological examination of ovaries revealed that the total follicle population at metestrus significantly ($P<0.05$) except in 2.4 ml buFF-treated animals where the percentage of atresia increased ($P<0.05$) as compared to control and other treated animals.

Follicular Fluid(FF) is reported to contain a number of non-steroidal compounds including inhibin (Henderson et., al., 1984) Administration of steroid free-bovine follicular fluid specially suppresses plasma concentrations of follicle stimulating hormone (FSH) and delays the onset of estrus in laboratory as well as farm animals (Miller et.al., 1979 ; Mc Neilly, 1984). Informations on effects of exogenous administration of bovine, porcine and ovine FF in other species are available in literature. Such attempts with buffalo follicular fluid (buFF) are extremely scanty. The present study, therefore, made an attempt to find out the influence of different doses of buFF administration on ovarian function in guinea pigs as the test animal.

MATERIALS AND METHODS

Mature female guinea pigs, weighing 400 to 600 g, were maintained at room temperature and fed normal diet supplemented with Vit, C and water ad libitum. Stages of the estrous cycle were determined every morning and evening (6 am, 6 pm) by inspection of the opening of vaginal membrane and its cytology as described by Garris and Foreman (1984). The day of estrus was designated as day 'O: Only those animals which had exhibited at least two consecutive regular cycles (16 ± 2 days) were considered for experiments.

Collection and extraction of follicular fluid:- Buffalo ovaries were collected from local abattoir and

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transported to the laboratory on ice. FF was aspirated from the follicles of all sizes visible on the ovarian surface using a hypodermic needle (22g) and syringe. In order to remove the steroids, activated charcol (Polypharm Pvt Ltd.,Bombay) was added @ 5 mg/dl to the FF and stirred at room temperature for 1h in a glass beaker. The mixture was centrifuged at 12,000g for 1h at 4°C. The supernatant was decanted aliquotted and stored at –20°C until use.

Experimental procedure Twenty four guinea pigs cycling normally were randomized into 4 groups (A,B,C and D) of 6 animals each. Animals in group A, B and C received 0.1 ml, 0.2 ml and 0.4 ml buFF, respectively twice daily in the morning and evening (6 am, 6 pm) for 3 days beginning on 9th of the cycle. However, the animals in group D (control) received equal volume of normal saline at times similar to that of other groups.

All animals were sacrificed at 24h after onset of estrus. After sacrifice, abdominal cavity was opened and both the ovaries were dissected, cleaned and weighed. Each ovary was examined under a stereo zoom-microscope and the ovulation points visible on the ovarian surface were counted.

Quantitative histology Since, mean frequency of ovulation in guinea pigs has been similar in both the ovaries (Norris and Adams, 1979) one every from each animal was randomly selected, fixed in formaline, dehydrated in a serial solutions of alcohol and embeded in paraffin. Serial sections of 5 um thickness were serially prepared from each overy using a microtome Every 7th section was mounted in rows on glass slides and stained with Ehrlich`s hematoxylin and eosin and examined under the microscope. An occular micrometer was used to measure the two largest diameters of the follicle at right angles and the diameter through a cross section containing the nucleolus of the oocyte. The sizes of the follicles were determined by averaging these diameters. Atretic follicles, which often lacked the nucleoli in the oocyte, were measured in a section containing the maximum nuclear diameter of the oocyte. Follicles measuring ≥ 200 um in diameter were counted and categorized into 4 size classes: I, 200 to <400 μm; II 400 to <600 μm; III 600 <800 μm; IV 800 to 1000 < μm Non-atretic (healthy) and atretic follicles were differentiated by the criteria described by Fujimori et al. (1987). The date was statistically analysed adopting standard procedure (Snedecor and cochran 1968)

RESULTS AND DISCUSSION

i. Onset and duration estrus: The data presented in Table 1 indicates that the treatment with buFF delayed the display of estrus in all animals. A dose related increase in onset of estrus following administration of buFF has also been reported for sheep (Henderson et.al 1986) and cattle (Quirk and Fortune, 1986) The mean duration of estrus in either of the buFF-treated groups was not significantly different from control. Ovarian weights at metestrus were also not affected by the treatment or dose of buFF. In our preliminary study it has been shown that administration of buFF in guinea pigs results in decline in the number of non-atretic follicles (Kumar, 1997). Thus,this implies that estrus

delay in these animals occurred due to reduce folli cular production of estradiol since, atretice follicles do not produce this hormone (Terranova, 1980) and behavioural estrus is an event, dependent upon large amount of estradiol produced by follicles attaining ovulatory maturity (Robinson, 1959).

II Ovulation rate: The mean ovulation rate in guinea pigs treated either with 0.6 ml or 1.2 ml buFF did not differ from that of control (Table 1) However, 50 percent animals in 2-4 ml buFF group failed to ovulate while remaining guinea pigs had ≤2 ovulations.

Since pituitary gonado trophins provide primary drive for ovarian follicular growth and development in several species including guinea pigs (Perry and Rowlands 1963) and buFF treatment suppresses FSH in a does dependent manner (Martin et.al., 1987) It can be presumed that the decrease of ovulation rate in higher does group probably related to a decline in circulating FSH and to the consequent decrease in the number of ovulatory follicles. This idea is supported by significant increas in percentage of atretic follicles in higher dose group.

III Follicle population: The total follicular population increased significantly (P<0.05) both in 0.6 ml as well as 1.2 ml buFF treated groups. (Table 2).This elevated number of class I and II or III follicles at metestrus in treated animals suggest that perhaps increase in FSH following with drawal of treatment stimulated growth in preantral and antral follicles and rescued some early-atretic large follicle Furthermore, in FF-treated rats, the initial decline of FSH is followed by an increase in its concentration (during 18-24h) after withdrawel of treatment (Noguchi et al., 1993)

Table 1. The Effet of treatment with three different doses of buFF on onset and duration of estrus, ovarian weight and ovulation rate in guinea pigs

Parameters	Experimental			Control
	0.6 ml buFF (group A n=6)	1.2 ml buFF (group B. n=6)	2.4 ml buFF (group C, n=6)	Saline (group D, n=6)
1. Interval from last FF/Saline injection to On set of estrus (d)	6.25 ± 0.11 *	6.39 ± 0.24 *	8.17 ± 0.75 **	5.25 ± 0.31
2. Duration of estrus (h)	9.67 ± 0.25	10.83 ± 0.40	9.00 ± 0.36	9.33 ± 0.40
3. Ovarian wt (mg) per 100 g of BW	16.87 ± 2.12	16.27 ± 1.08	15.15 ± 0.85	15.28 ± 1.14
4. Ovulation rate (CL)	2.83 ± 0.83	2.34 ± 0.61	0.83 ± 0.40 **	2.83 ± 0.30

Value represent the Mean ± SE of each group FF/saline given on day 9 to and 11 of the cycle ** P<01and 11 of the cycle ** (P<D, 01),*(P<0.05) different from control.

Table 2. Mean number (± SE) of follicles in various classes at metestrus in guinea pigs treated with three different doses of buFF.

Histological Class (μ m) Overall	Control (group D,n=6)	buFF treated 0.6ml (group A,n=6)	1.2 ml (group B,n=6)	2.4ml (group C,n=6)
H	1.00 ± 0.51	1.16 ± 0.65	1.33 ± 0.61	0.33 ± 0.33
A_1	4.83 ± 0.90	5.00 ± 1.54	7.00 ±1.41	4.00 ± 1.90
A_2	5.66 ± 0.84[a]	7.83 ± 1.17 [ab]	10.16 ± 1.62 [b]	5.33 ± 1.00[a]
A_3	2.33 ± 1.17	6.00 ± 1,92	3.33 ± 0.76	6.00 ± 1.75
Total	13.83 ± 0.94 [a]	20.00 ± 2.22 [b]	21.83 ± 1.13[b]	16.00±[a]1.41[a]

H-Healthy, A1 Early altretic, A2= Mid=mid-atratic, A3 Late-atretic Figures with in rows with different superscripts are different ($P<0.05$)

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Studies On some Biochemical Attributes of Cervical Mucus and Blood serum in Cows of Himachal

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ABSTRACT

The mean values of Calcium, inorganic phosphorus, glucose and total protein in cervical mucus (CM) vs blood serum during estrus were 5.04 ± 0.28 vs 6.38 ± 0.18 mg/dl, 1.12 ± 0.15 vs 3.68 ± 0.26 mg/dl, 1.02 ± 0.32 vs 52.23 ± 2.38 mg/dl and 0.57 ± 0.12 vs 9.35 ± 0.2 mg/dl respectively. The levels of all these biochemicals in CM were significantly lower ($P<0.01$) than in blood serum, Calcium, inorganic phosphorus and glucose exhibited a positive correlation (r = 0.07, 0.37 and 0.16 respectively), whereas total protein had a negative correlation of 0.10 between CM and blood levels. All these correlations were non-significant.

The significance of various biochemical constituents of cervico-vaginal and uterine fluids has been well recognised, as their deficiency or excess adversaly affect the viability and fertilising ability of sperms (Hidiroglou, 1975). Scanty information is available on the concentration of different biochemical constitutents in CM of cows in relation to the blood levels, The present study was therefore carried to evaluate the concentration of calcium, inorganic phosphorus, glucose and total protein in CM and blood along with their correlation, in cattle of Himachal Pradesh.

MATERIALS AND METHODS

Twenty one adult, healthy, cycling cows (Local and Local x Jersy) presented during estrus to Veterinary Clinics of H.P.K.V.Palampur, were used for the study, Presence of normal genitalia and estrus were confirmed, All the animals had moderate to good uterine tone and CM was clear, copious, stringy and exhibited typical fern pattern, Before these animals were inseminated, the CM was collected aseptically. Blood was collected by jugular venepuncture, allowed to clot and serum was removed. All the samples were preserved at – 20°C till analyses. The biochemical estimations were carried out with the help of AMES CH 100 SEAC semi – auto analyser (Miles IndiaLtd.,Baroda) .Statastical significance was determined by students "t" - test (Gupta, 1969).

RESULTS AND DISCUSSION

Calcium: Mean level of calcium in CM was 5.04 ± 0.28 mg/dl which was significantly lower (P<0.01) than the corresponding value of 6.38 ± 0.18 mg/dl in blood serum, respectively. Similar levels of calcium in CM and blood of buffalo cows were reported by Takkar et.al. (1992), whereas Bhosrekar et.al. (1995) have reported lower calcium levels in CM of cows. The calcium levels in CM were found to be positively correlated (r ± 0.07) with calcium in blood, but without any statistical significance, Calcium dependent mechanisms are involved in steroid biosynthesis in ovaries and LH release from pituitary (Hurely and Doane, 1989) It is one of the major cation in CM which stimulates glycolysis thereby sustaining the viability, motility and metabolism of the sperm (Sidhu and Guraya 1985)

Inorganic phosphorus: The inorganic phosphorus content in CM was significantly (P<o.01) lower than in blood serum, with a mean value of 1.12 ± 0.15 mg/dl and 3.68 ± 0.26 mg/dl respectively, Almost similar values of inorganic phosphorus have been reported in CM of crossbred cows by a number of workers (Goel et.al., 1974) ; Wani et.al ., 1979 ; Krishnaswami and Uthappa, 1984). However, slightly higher values of inorganic phosphorus in blood serum were reported by Kumar and Sharma (1991), whereas Enkhia et.al (1983) reported slightly lower values, There was a positive correlation (r ± 0.37) of inorganic phosphorus between CM and blood serum. Inorganic phosporus has been reported to be essential for energy transformation cellular level and is associated with maintenance of sperm glycolysis and respiration (Krishnaswami and Uthappa, 1984).

Glucose: The levels of glucose in CM and blood serum of cows were 1.02 ± 0.32 mg/dl and 52.23 ± 2.38 mg/dl respectively, the values being statistically significant (P<0.01).It was however interesting to note that the glucose levels in CM of six animals were undetectable, There was no available literature regarding glcose levels in CM except for Sidhu and Guraya (1985) who have reported very little sugar in cervico-vaginal fluid. The blood levels of glucose were within normal limits (Kumar and Sharma, 1991). Glucose in CM exhibited a positive correlation (r = 0.16) with circulating levels in blood. Blood glucose has been reported to affect conception as it can be utilised as a source of energy for sperms (Goel et.al., 1974)

Total protein: mean value of total protein in CM (0.57 ± 0.12 mg/dl) was significantly lower (P<0.01) than that in blood serum (0.35 ± 0.2 md/gl). However, total protein in CM has been reported to be quite high (Wani et.al., 1979). In blood serum almost similar values have been reported by Gandhotra et.al. (1993). Whereas Kumar and Sharma (1991) reported lower values. Total protein in CM shared a negative correlation (r ± 0.10) with the blood levels. Protein in CM improves sperm transport, Apart from this, proteins also regulate the osmolarity, consistency threadibility and buffering capacity of cervical mucus (Goel et.al 1974).

From the correlation of the above mentioned bio-chemical in CM and blood, it could be postulated that CM is not only the product of simple diffusion but also of active secretions, as been reported earlier by Takkar et.al (1992). Correlation of these bio-chemical constituents in CM and blood with conception rate need to be investigated further.

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Studies on some factors influencing treatment and maternal Recovery rate in uterine torsion among crossbred dairy cattle

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ABSTRACT

Correction of torsion by manual rolling of the dams and subsequent delivery per vagina was successfully employed in 40.6 per cent of the case of uterine torsion. While the manual rolling of the dam was most successful in cases referred within 24 hours after the onset of labour, the success rate declined drastically in cases presented later than 24 hours. The overall maternal recovery rate in case of uterine torsion relived by manual rolling of the dam was 84.0 per cent. About 59 per cent of cases of uterine torsion were considered irreducible and were relieved by laparo hysterotomy with maternal recovery rate of only 37 per cent.

Torsion of the uterus has been recognised as the most predominant cause of maternal cause of maternal dystocia in dairy cattle. The purpose of this investigation to analyse the factors which influence the choice of treatment and subsequent recovery rate of the dams affected with uterine torsion.

MATERIAL AND METHODS

The clinical records of uterine torsion available in the department of Gynaecology and Obstertrics, Veterinary College Bangalore between 1981 and 1992 were analysed with reference to the gestational length at the time of presentation of the cases, the viability and sex of the fetus and the position of uterine torsion. Information regarding approximate duration of dystocia was also obtained from the clinical records and based on this, the animals were classified into those referred as within 12 hours, 12-24 hours, 24-48 hours and 48 hours after the onset of labour.

The records were also screened to analyse the treatment procedures adopted to relieve uterine torsion and an attempt was made to correlate the choice of treatment preferred in relation to duration of dystocia and subsequent recovery rate of the dams.

RESULTS AND DISCUSSION

A total of 140 obstetrical cases were referred to this clinic between 1981 and 1982, 34 of which were considered to have occurred due to uterine torsion. 46 of the 140 cases were considered to be the cases of maternal dystocia, uterine torsion alone accounting for 73.91 per cent of these cases. Although, these figures appear to be rather high, there appears to be a definite geographical variation in the incidence of dystocia. Further, the figures reported in the present investigation may not exactly represent the overall incidence of dystocia in crossbred cows as they

comprised of only those cases referred to this clinic following unsuccessful attempts by the local Veterinary personnel.

Twenty eight of the 34 cases of uterine torsion referred to the clinic were presented at term and the rest between seven and 9 months of gestation The incidence of post-cervical torsion was higher (73%) than pre-cervical torsion (27%) and is in agreement with the observation of Pearson (1971). Available records indicate a tendency for uterine torsion to be associated more with male fetus (n=9) as against female fetus (n=6). Similar observation have been made by Pearson (1971). Pattabiraman et.al (1979) recorded a high incidence of male fetus (68.7%) in uterine torsion.

Torsion was relieved following manual rolling successfully in all the six cases presented earlier to 24 hours after the onset of labour. Whereas, only 75 % and 66 % of cases could be relieved when presented after 24 and 48 hours respectively. Pattabiraman et. al (1979) also made similar observation and concluded that detorsion was unsuccessful in cases presented after 3 days.

The overall success rate by manual rolling of the dam was 40.60 per cent and is much lower than 61.00 to 68.00 per cent reported by Pearson (1971) who concluded that manual correction procedures were more difficult if the fetus was dead. In the present investigation, a live fetus was encountered in only 3 of the 34 cases of uterine torsion and may possibly account for a lower success rate with manual rotation procedures.

Torsion of the uterus was found to be irreducible in 19 (59.30 %) of the 32 cases and hence, were subjected to caesarean section. The maternal recovery rate following caesarean in cases of irreducible uterine torsion, was considerably lower (37.00%) and is in sharp contrast to the maternal recovery rate of 95.00 per cent reported by Pearson (1971). Pattabiraman et al (1979) reported 97% maternal survival rate following non-surgical correction of uterine torsion in contrast to 61.5 % after caesarean section. However, in the present investigation that the rate of maternal recovery was considerably lower if the animals were subjected to caesarean after suffering from torsion for 24 hours or more. The survival rate of dams, therefore, appears to depend upon the duration for which the torsion was existing, besides on the factors such as degree of torsion and the extent to which the case was handled for manual reduction of torsion.

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Efficacy of Exapar for the expulsion of Placenta and as Uterine Tonic in cows and Buffaloes

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ABSTRACT

Administration of Exapar alone in 10 cows and 4 buffaloes and with antibiotic cotherapy in further two cows and one buffalo, helped in the timely expulsion of placenta in all (100 %) of the uncomplicated cases. In fastly adherent retained placenta cases (6 cows and 4 buffaloes) Exapar with or without antibiotic facilitated the manual removal of placenta. The treatment also resulted in improvement of post parturient parameters in the animals. The conception rate of treated cows and buffaloes was 72% and 55% as against 40% and 25% respectively in the untreated healthy animals of the two species.

This communication deals with the efficacy of a herbal uterine cleanser and tonic in the expulsion of placenta and in improving the post-parturient reproductive performance of cows and buffaloes.

MATERIALS AND METHODS

The study was carried out at the dairy farm_ of IAAS Rampur Chitwan as well as in adjacent private diaries. A total of 27 animals comprising 18 cows (Holstein Friesian, Chersey X) and 9 buffaloes (Murrah X and Local) in 1st to 6th calving were included in the study on the basis of non-expulsion of placenta within 12h of parturition. The animals were assigned to the following groups according to treatment regimes given at parturition:

A1. Simple cases (10 cows and 4 buffaloes treated with exapar alone @ 100 ml twice a day on the first day and 50 ml twice daily for the next 3 days.

A2. Simple cases (2 cows 2 buffaloes) treated with Exapar as in A1 alongwith Antibiotic therapy.

B1. Complicated cases with fastly adherent placenta requiring manual removal of placental treated with Exapar alone as for A1 (3 cows and 2 buffaloes)

B2 Complicated cases, with fastly adherent placenta requiring manual (removal) treated with Exapar as well as some antibiotic (3 cows and 2 buffaloes)

C. Nine other parturating animals (5 cows and 4 buffaloes) were kept untreated as Healthy contorl.

The effect of medication on expulsion of placenta and subsequently on the post-partum reproductive health of the animals was recorded.

Expar as Uterine tonic in buffaloes In order to further validate the efficacy of Exapar as uterine cleanser–tonic, ten buffaloes (Murrah X and Local breeds) in 1st to 4th calving were selected. While 5 buffaloes were treated with Exapar 100 ml twice a day on the first day followed by 50 ml twice daily for the next 3 days, the other 5 were retained as untreated control.

RESULTS AND DISCUSSIONS

Administration of Exapar alone appeared to have helped in the expulsion of placenta in all (100%) of the uncomplicated cases viz., 10 cows and 4 buffaloes. All expulsions in such cases were effected within 24 hours of calving which indicated the efficacy of Exapar therapy as a cleansing draught. Moreover, in complicated cases Exapar with or without antibiotic cotherapy appeared to facilitate the manual removal of the placenta. The treatment also appeared to have offset the likely adverse effect of retained placenta on the various post-parturient parameters of these, the beneficial effect was maximum in the conception rate as 72% of the treated cows 55% of the treated buffaloes conceived as against 40% and 25% respectively in the untreated healthy animals of the two species. Even among the repeat breeder cows and buffaloes following retained placenta 62.5% were confirmed pregnant following post-partum oestrue in the cows with retained placenta treated with Exapar was only slightly delayed (30-60 days) as compared to the healthy cows (20-60 days) whereas in buffaloes there was no difference in this regard between the treated and healthy animals. The milk yield in the treated animals was not adversely affected.

The efficacy of Exapar as uterine tonic was further studied in parturating buffaloes. The results indicated that apart from accelearating the explusion of the membranes, treatment led to early onset of post-partum estrum (16 to 30 days) as compared to 30 to 60 days in the healthy control animals. Further 80% of the treated buffaloes were confirmed pregnant as against only 40% found pregnant in the control .Exapar a product Dabur Ayurvat Ltd., is a combination of herbs. Some earlier reports have validated its efficacy in expulsion of membranes, restoration of lochial discharge and involution of uterus in bovines (Signal, 1996) and in improvement of reproductive efficiency in buffaloes (Khanna et. al., 1997). The present results are in agreement with these reports.

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Biochemical changes Associated with Retention of Fetal Membranes in Buffaloes*

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ABSTRACT

Studies were conducted on 30 rural buffaloes, suffering from retention of fetal membranes (RFM.) Serum glucose, total protein, calcium and inorganic phosphorus levels were estimated on 1st and 30th day post-partum and compared with that of controls (parturition without RFM). Serum levels of all the referred biochemical estimates were significantly (P<0.08) lower, in buffaloes having RFM, as compared to buffaloes of control group on 1st and 30th day post-partum. On comparing the manual and non-manual removal groups, it was found that, the serum levels of all the biochemical constituents were higher, On day 30th post-partum, in non-manual groups. Five different therapeutic regimens were followed and their comparative efficacy with relevance to biochemical changes were analysed.

Retention of fetal membranes is one of the most common post-pertum complications in dairy animals. Role of certain biochemical constituents viz. Glucose, serum total protein and microminerals in various reproductive processes have been well documented (Quanyam et.al.,1985).

Dutta and Dugwekar (1983). And Choundhary et.al., (1993), also reported relationship of certain serum biochemical constituents in buffaloes.

Since the rural buffaloes are more prone to nutritional mismanagement was envisaged to under take the serum biochemical studies associated with RFM in them.

MATERIALS AND METHODS

To study the biochemical changes, associated with RFM, blood samples were taken from each of 36 buffaloes (30 buffaloes, retaining their fetal membranes and 8 control). Buffaloes with RFM were subjected to five therapeutic regimens. In group I, manual removal of RFM was done along with intra-uterine administration of tetracycline hydrochloride daily till normal lochiae appeared. Group II, III, IV and V were non manual removal groups. Group II and III were treated with parentral oxytocin whereas group IV and V animals were given ergometrine maleate, parentrally. In addition to

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ecbolics, group III animals were also given oxytetracycline, parentrally, whereas group II and V animals were infused with oxytetracycline intra uterine. First blood sample was taken before the commencement of therapeutic measures, on Ist day and the second, on 30th day, after therapeutic measures commenced.

Approximately 30ml of blood, from each buffalo, was collected from jugular vein, in 50 ml sterilized glass tubes. The serum was separated and kept in sterilized vials. Glucose and total protein were estimated. Immediately after the separation of serum and the remaining serum was stored at – 20°C until analysis of calcium and inorganic phosphorus.

Estimation of different biochemical parameters viz., serum glucose total proteins ,serum calcium and inorganic phosphorus were done adopting standard procedure (Doumas et.al,1971 and Tietz.1976)

RESULTS AND DISCUSSION

The mean values of serum biochemical estimates in control and RFM group of buffaloes on Ist day and 30th day post-partum are presented in Table 1.

The mean value of Serum glucose in buffaloes with RFM was significantly (P<O.05) lower (51.00 ± 55 mg %) than the normally calved buffaloes (61.86 ± 1.17 mg %) On Ist day postpartum. It also differed significantly (P<0.05) on day 30th post-partum (46.55 ± 0.57 mg % vs 53.25 ± 1.59 mg %) Similar trend has been reported by Dutta and Dugwekar (1983) and Choudhary et. al., (1993). In buffaloes with RFM and without RFM. The lower level of glucose leads to atony of uterine tissue and weak uterine contractions, causing delay in the process of parturition (Mohanty et. al., 1994) and might be attributing to RFM.

Further, level of serum glucose (Table 2) in the buffaloes of manual removal group, was significantly (P<O.05) lower (43.10 ± 1.33 mg %) as compared to those of non-manual removal group (47.41 ± 0.52 mg %)

Amongst non-manual removal groups (Table 3) in group V animals serum glucose level was almost at par control (49.00 ± 1.14 mg % vs 53.25 ± 1.59 mg %). However, non-manual removal group buffaloes were having significantly (P<0.01) lower serum glucose level than the control animals. The trend in group V animals suggest that the metabolic activity was at par control group. Hence it can be presumed that this treatment (group V) was better in terms of restoring the energy levels.

The mean value of serum total protein on Ist day post-partum, was significantly (P<0.05) lower (8.0 ± 0.0 g%) in buffaloes with RFM, as compared to normal parturient buffaloes (6.32 ± 0.07 g %) On 30th day post-partum, the mean value were 6.48 ± 0.07 g percent and 7.01 ± 0.07 g percent in animals with RFM and normal parturition respectively (Table 1) and differed significantly (P<0.05).

The results of the present study are consistent with the reports of Dutta and Dugwekar (1983) in buffaloes.

However, Choudhary et al., (1993) did not find any significant variation in serum total protein level in RFM buffaloes.

Further, the level of serum total protein in manual removal group (group I) was significantly (P<0.05) lower (Table 2) than in non-manual removal group animals (5.88 ± 0.06 vs 6.63 ± 0.05 g %) Amongst non manual treatment group (Table 3) group V buffaloes attained the serum total protein level at par to control group. However, in group II, III and IV the level was significantly (P<0.01) lower than that of the control animals.

The mean value of serum calcium, in buffaloes with RFM, on1st day post-partum, was significantly (P<0.05) lower (Table-1) (8.24 ± 0.08 mg %) than in the control animals (9.85 ± 0.24 mg %). The corresponding mean value on 30th day post-partum, was significantly (P<0.05) lower (8.94 ± 0.07 mg %) in buffaloes with RFM than the normally calved buffaloes (10.20 ± 0.33 mg %) Dubas et al. (1987) in Murrah Buffaloes. Pathak et.al., (1991) In Surti buffaloes and Mohanty (1994) in bovines, Also observed similar pattern.

Further, serum calcium level in manual removal group (Table-2) animals, was significantly (P<0.05) lower (8.54 ± 0.10 mg %) than the non-manual removal group (9.04 ± 0.07 mg %) signifying that the non-manual treatment is better than the manual, in terms of restoring the calcium level. Amongst non-manual treatment groups (Table-3) the level of serum calcium was significantly (P<0.01) higher in group III and group V buffaloes, in comparision to that of group II however, not at par control.

The mean value of serum inorganic phosphorus, of Ist day post-partum, was significantly (P<0.05) lower (4.07 ± 0.03 mg %) in animal with RFM, as compared to those with normal parturition (5.17 ± 0.14 mg %). Similarly, the corresponding mean value in both of the above groups also differed significantly (P<0.05) on 30th day post-partum (Table-1) (4.35 ± 0.30 vs 5.26 ± 0.14 mg %) Similar trend has also been observed by Shukta et.al., (1983) in Gir cows and their crosses and Dabas et.al., (1987) in Murrah buffaloes. However, Choudhary et.al., (1993) could not find, appreciable changes in the level of inorganic phosphorus, in rural Murrah buffaloes, retaining their fetal membranes.

The level of serum inorganic phosphorus was non-significantly different (Table-2) in manual removal group than the non-manual normal group. Further, amongst non-manual removal groups (Table-3) none of the groups could attain the level of serum inorganic phosphorus at-par control animals.

Table 1.Serum biochemical constituents in control/RFM buffaloes on Ist/30th day Post-partum

S.No.	Biochemical	Mean ± S.E			
		I st day post-partum		30th day post-partum	
		Control (6)	RFM (30)	Control	RFM
1.	Glucose	61.86[a] ±1.17	51.00[b] ±0.55	53.25[a] ±1.59	46.55[b] 0.57
2.	Total Protein	6.32[a] ± 0.07	5.00[b] ±0.03	7.01[a] ±0.07	6.48[b] ±0.07
3.	Calcium	9.85[a] 10.24	8.24[b] ±0.08	10.20[a] ±0.33	8.94[b] 0.07
5.	Inorgtanic Phosphorus (mg %)	5.17[a] ±0.14	4.07[b] ±0.03	5.26[a] 0.14	4.35[b] 0.30

Figuers in parenthes is indicate number of buffaloes

Table 2 Serum biochemical constituents with reference to type of therapeutic Regimens on 30th day post-partum

S.No	Biochemical Constituents	Mean ± S.E. Types of therapeutic regimens		
		Control	Manulremoval	No-manualremoval
1.	Glucose (mg %)	53.25[a] ±1.59	43.10[c] ±1.33	47.41[b] ±0.52
2.	Total protein (g %)	7.01[a] ±0.07	5.88[c] ±0.08	6.63[b] ±0.05
3.	Calcium (mg %) (mg) %	10.20[a] ±0.33	8.54[c] ±0.10	9.04[b] ±0.07
4.	Inorganic phosphorus (mg %)	8.26[a] ± 0.14	4.30[c] ±0.04	4.38[b] ± 0.03

Means having different superscripts, differ significantly

Table. 3 Group wise serum biochemical constituents to buffaloes on 30th day post-pertum

S. No	Biochemical Constituents	Group - Mean ± S.E.					
		Control	I	II	II	IV	V
1.	Glucose (mg %)	53.25^{a} ±1.59	43.10^{c} ± 1.33	45.11b^{c} ±0.60	47.59^{b} ±0.94	47.91b ±0.47	49.00^{ab} ±1.48
2.	Total Protein (g %)	7.01^{a} ± 0.07	5.88^{c} ± 0.06	6.39^{c} ± 0.04	6.50^{c} ± 0.00	6.72^{b} ± 0.08	6.90^{b} ± 0.07
3.	Calcium (mg %)	10.20^{a} ± 0.33	8.54^{c} ±0.10	8.59^{c} ± 0.82	9.20^{b} ± 0.09	9.08^{bc} ± 0.09	9.29^{b} ± 0.04
4.	Inorganic Phosphorus (mg %)	5.26^{a} ± 0.14	4.30^{b} ± 0.04	4.32^{b} ± 0.04	4.34^{b} ±0.05	4.37^{b} ±0.05	4.42^{b} ± 0.08

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Serum Total Protein and total Cholesterol Levels in Mahsani Buffaloes Retaining Fetal Membranes*

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ABSTRACT

The serum total protein was significantly higher in the group of buffaloes retaining fetal membranes after abortion as compared to control group. Serum globulin was significantly higher in RFM groups as compared to control. RFM buffaloes had low A::G ratio as compared to control group with the difference being non-significant. Serum cholesterol was significantly higher in the group of buffaloes retaining fetal membranes after parturition as compared to control.

Retention of fetal membrane is a common post-partum and post abortion complication which adversely affects the reproductive performance in buffaloes and results in great economic losses. The biochemical aspects of etiology and pathogenesis of retained fetal membranes after parturition is incompletely understood and hence the present study was undertaken to study the levels of protein and total cholesterol in the affected and control buffaloes.

MATERIAL AND METHODS

The study was undertaken on Mehsani buffaloes of Mehsana district. Gujarat. Buffaloes were grouped in three different groups.

Viz., Group-I 10 buffaloes not retaining fetal membranes after normal parturition (WRFM or control)

Group-II 25 buffaloes retaining total membranes after parturition (RFM/AP)

Group-III 11 buffaloes retaining fetal membranes after abortion (RFM/AA)

The animal was considered to retain fetal membrane when placenta was not expelled within 12 hours after parturition or abortion. Blood was collected prior to treatment in both the RFM groups and after the placental expulsion in the control group. Serum total protein, albumin and total cholesterol were

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estimated using Diagnostic Reagent kits (Span Diagnostics Ltd Udhna, Gujarat).

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

RESULTS AND DISCUSSION

The mean serum total protein was found to be 6.93 ± 0.24, 7.73 ± 0.25 and 8.23 ± 0.19 grams per 100 ml of serum in the Group-I, Group-II, and Group-III respectively The difference was non-significant between the Group-II and control but was significantly differing (P<0.05) between Group-III and control (Table-1) Overall the total proteins were higher in both groups of animals retaining placenta as compared to control group. The observed higher levels of proteins in animals retaining placenta could be attributed to higher levels of globulin as compared to control group of animals Serum globulin was significantly (P< 0.01) higher in RFM groups as compared to control. An increase in globulin for the Group-III gets support of Kolev et.al. (1977) who reported an increase in the alpha and Beta globulin contents of the blood on the day of abortion. In the present study, Group-III had higher level of albumin as compared to rest of the two groups with non-significantly differing values. This has resulted in to A:G ratio of 1.14 ± 0.15 0.88 ± 0.07 and 0.92 ± 0.06 for the Group-I, Group-II and Group-III respectively. But the difference between the groups were non-significant (Table –1).

The mean serum cholesterol values were 106.00 ± 4.52, 155.20 ± 10.55 and 135.45 ± 6.52 mg per 100 ml of serum in Group-I, Group-II, and Group-III respectively. The difference was significant (p< 0.05) between the Group-II and control but non significant between the Group-III and control group (Table-1) Mean total cholesterol was found to be higher in both the RFM groups as compared to control. A decline in cholesterol level at parturition may be due to depletion of cholesterol reserves as cholesterol is the precursor of steroids which increase considerably near parturition stage M.C Donald, 1980) This explains the low levels of total cholesterol found in the control group buffaloes. Higher level of total cholesterol in the Group-II might be due to the impairment in the production of steroids and especially the circulating estrogen might not be produced in sufficient amount necessary to expel the place

Table 1 : Average of certain serum Biochemical Constituents of Buffaloes retaining fetal membranes.

Parameters	GROUPS		
	Group-I WRFM N=10	Group-II RFM/AP N=25	Group-III RFM/AA N=11
Total Protein	6.93 ± 0.24[a] (6.26 – 7.92)	7.73 ± 0.25[ab] (5.37 – 9.46)	8.23 ± 0.19[b] (6.90 – 9.46)
Albumin	3.53 ± 0.21 (2.73 – 4.68)	3.40 ± 0.12 (2.03 – 4.29)	3.89 ± 0.15 (2.93 – 4.68)
Globulin	3.28 ± 0.35[a] (1.44 – 4.80)	4.37 ± 0.24[b] (1.89 – 6.40)	4.34 ± 0.20[b] (3.63 – 5.75)
A : G ratio	1.14 ± 0.15	0.88 ± 0.07	0.92 ± 0.06
Total Cholesterol	106.00 ± 4.52[a] (90 – 120)	155.20 ± 10.55[b] (90 – 240)	135.45 ± 6.52[ab] (110 – 180)

i. Figures in the parentheses indicate range;
ii. Means bearing different superscripts differsignificantly.

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Effect of sex and type of kidding on gestation length and birth weight in black Bengal goat

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ABSTRACT

Fifty kiddings of Black Bengal goats were studied for gestation length, sex and type of kidding and birth weight of kids for 24 months. Statistical analysis revealed that there was no significant difference in gestation length neither between and female kids, nor between single and twin kidding. The birth weight of single and individuals of twin kidding were non significant. However, a highly significant difference ($P < 0.01$) was observed between the birth weight of male and Female kids.

Birth weight and gestation length are the most observable characters in the farm animals. In animal breeding, improvement can be done by selection and birth weight is the first observation which can be criteria to select superior animals for improved production. Moreover, birth weight determines the prospective value of the animal in the later part of their life through physical and functional development. Also, gestation length is the important observable character is domestic animals. Both the birth weight and gestation length are influenced by number of factors. In Tripura, mostly Black Bengal goat and few Assam Hill breed are kept for meat purpose but their birth weight and gestation length were not adequately studied in Tripura. In the present study, observations on the effect of sex and type of kidding on gestation length and birth weight of Black Bengal goats were studied.

MATERIALS AND METHODS

Fifty kiddings of Black Bengal goats were studied for gestation length, sex and type of kidding and birth weight of kids in our institute farm for 24 months. The gestation length was calculated from the date of fertile natural service to the date of kidding. The birth weight and sex of the kid was recorded soon after their birth. Standard feeding and managemental practices were adopted throughout the period under study. Data collected were analysed statistically (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

Out of fifty kiddings studied, 22 (44%) were single, 27 (54%) were twin and 1 (2%) was triplet kidding. Number of male and female kids born were 35 (44.3%) and 44 (55.7%) respectively. The mean gestation length for male

kids (146 + 0.245 days) was more than the female kids (144.00 + 0.204 days) Similarly, gestation length for single birth (144.91 + 0.267 days) was more than twin (144.34 + 0.267 days) and triplet (143 days) birth. The mean birth weight of the male kid (1.164 + .004 kg) was more than female kid (1.064 + .008 kg). The mean birth weight of single kid (1.132 + 0.013 kg) was more than that of individuals of twin (1.101 + 0.008 kg) and triplet (1.077 + 0.051 kg) kidding. Statistical analysis revealed that there was no significant difference in gestation length neither between male and female kids, nor between single and twin kidding. The birth weight of single and individuals of twin kidding was non significant, However a highly significant difference ($P < 0.01$) was observed between the the birth weight of male and female kids irrespective of type of kidding. Verma et. al., (1990) reported the similar results but the result was statistically non-significant which might be due to the difference in breeds. Almost similar observations have been reported by Badawy et al. (1972) In Angora breed and Das and Tomar (1980) in crossbreed goats, More birth weight in the male kid may be attributed to its greater gestation length. This might be due to the fact that male gonad gets activated earlier than the female gonad. The secretion of male sex hormone have an anabolic effect, which results into faster growth of male foetus during prenatal development (Hafez, 1980). Tiwari et.al., (1969) postulated that the individuals of twin kidding might be lighter than the single born kids because of limitation of the uterine environment.

Therefore, it can be concluded that gestation length was not affected by sex and type of kidding. Also, birth weight was not effected by type of kidding but by the sex of kids.

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Studies on Incidence of Canine Endometritis in Bhubaneswar City*

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ABSTRACT

One hundred sixty seven bitches with Gynaeco-Pathological problems were screened, out of which 28 74 per cent cases were found to be affected with endometritis. The occurrence in older animals was higher than young ones. The overall body temperature (102°F) was not markedly elevated Mucopurulent (47.91per cent) and Muco-sanguinous (20.83 per cent) were predominant uterine discharge Marked in appetence was seen in majority of cases (60.50 per cent).

Endometritis in bitch is common anomaly which usually follows post-oestrual or post-coital course which is supposed to be hormonally mediated. Diverse clinical manifestations have been recorded by various workers. In majority of reports elaborate gyaneco-clinical examination picture is lacking. The present investigation has been aimed to study the incidence and related Physico-gynaecological picture in Canine endometritis.

Part of M. V.Sc thesis of first author submitted to Orissa University of Agricultural Technology Bhubaneswar- 751 003

MATERIALS AND METHODS

The present study was undertaken in the Department of Gynaecology, Orissa Veterinary College, Bhubaneswar during the period from 1995 to 1997. A total number of 167 cases were screened for various reproductive disorders out of which 48 bitches of diverse genetic group (Alsatian, Doberman, Labrador and Spitz) have been gynaeco-clinically investigated. Age, nature of genital discharge, body condition, feeding habits, previous breeding history and body temperature were taken into consideration.

RESULTS AND DISCUSSION

During the present study 167 bitches with Gynaeco-pathological problems were investigated and overall 48 (28.74 per cent) of cases were identified with endometritis after careful Physico-gynaecological examination, A maximum of 39.03 per cent was recorded during 1997 and the incidence was lowest in 1995 (20 96 per cent) The present overall incidence is in partial agreement with Roberts (1982) who recorded 16.3 per cent, however Gandotra et al (1993) reported a lower incidence. The discrepancy might be due to variation in the breed, age

management and type of data processing. The overall incidence of the disease was found to be more prevalent (62 50 per cent) at more than 6 years of age and between age group of 3-6 years it was 23.91 per cent. Animals of 1 to 3 years of age had lowest occurrence (14.58 per cent) Similar observations has been made by De Coster et al (1979) De Fanti et al (1992) In Canines endocrine disturbances have been attributed as a major factor for causing endometritis. The increased frequency of this condition in old age might be due to aberration of endocrine function. The overall body temperature of endometritis case was (102.2°F) The present finding is in consonance with Roberts (1982) and Gandotra et al (1994). Minor elevation of body temperature recorded in the present study might be due to moderate systemic reaction or production of insufficient quantity of toxins which failed to evoke pyrexia.Mucopurulent discharge (47.91 per cent) was predominant uterine discharge followed by sero-sanguinous of 20.83 per cent in bitches with endometritis which supports the findings of Brodey and Fidler (1966) and Allen and Renton (1982) Mucoid discharge was seen in 31.25 per cent of cases. The abnormal uterine discharge from affected bitches might be due to interaction of uterine bacteria with concurrent local immunological response resulting in death of WBC and RBC.

Majority of affected (62.5 per cent) cases had depraved appetite which was in agreement with Borresen (1980) and Mayers Wallen et al (1986) General body condition was either fair of poor (41 67 per cent) in majority of cases while only 16.66 per cent of cases had good body condition. This observation corroborates the finding of De Coster et al (1979) and Poweda and Harendra Kumar (1996) This might be due to malfunction of liver, kidney resulting from mild toxaemia.

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Studies on some Enzymes levels and their extra-cellular leakage during preservation of punganur bulls semen

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ABSTRACT

The overall mean activities of Alkaline phosphatase (AKP) Acid phosphatase (ACP) and Lactic Dehydrogenase (LDH) enzymes in the neat seminal plasma of Punganur bulls were 584.87 ± 11.22 KAU/100 ml, 241.47 ± 2.31 KAU/100 ml and 356.22 ± 9.45 IU/liter,respectively. A nonsignificant ($P>0.05$) difference was noticed between bulls and also between semen collections in the levels of all the three enzymes assayed. A significantly higher leakage of all the three enzymes viz., AKP, ACP and LDH was observed in the semen preserved with Egg Yolk citrate extender when compared to Tris extender. A significantly ascending order of extra-cellular leakage of enzymes was noticed with the increase in stroage time in both the extenders. A highly significant ($P<0.01$) difference was also noticed between bulls in leakage of AKP and ACP but nosignificant with respect to LDH.

Enzymes viz., Alkaline phosphatase (AKP) Acid Phosphatase (ACP) and Lactic dehydrogenase (LDH) were reported to have important role on the functional status of the accessory sex glands, epididymis and ampullae (Ibrahim et al. 1985) and their effect on the keeping quality, sperm metabolism and fertility (Belorkar, 1987) Hence, the present study was undertaken to investigate the levels of different enzymes in neat seminal plasma and their extra cellular leakage during preservation in Punganur bulls semen.

MATERIALS AND METHODS

The present study was undertaken at cattle farm, Livestock Research Station, Palamaner, A.P and at College of Veterniary Science, Tirupati Eighteen enjaculates from three Punganur bulls were collected. After assessing the motility, the neat and diluted semen in Tris and Egg Yolk Citrate (EYC) extenders at 0,24, and 48 hours of preservation was centrifuged at rate of 5000 rpm for 10 minutes. The seminal plasma was separated and transferred into sterile glass vials, labelled and stored in deep freeze until enzymatic assay carried out.

The three enzymes viz., AKP, ACP, and LDH were estimated in neat semen and diluted semen at 0,24, and 48 hours of preservation by using span

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Diagnostic kits. Statistical analysis of data was carried out by adopting the methods described by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The overall mean activities of AKP, ACP and LDH enzymes in neat seminal plasma of Punganur bulls observed in the present study (Table) was comparable with the values reported by Dhami and Kodagali (1988) in Surti buffalo seminal plasma. On the contrary, higher values of AKP and ACP were reported inbuffalo bulls by Chaudhary and Gangwar (1978) and Reddy and Raja (1980) respectively.Extra-cellular leakage of enzymes during preservation

The trend of extra cellular leakage of AKP and ACP enzymes observed in the present study (Table) was comparable to Vyawanare et.al (1989) and Azawi et.al (1990). However, the present findings could not be compared with the above mentioned authors as they have reported the values of AKP and ACP in different units.

The mean LDH activity at 0,24 and 48 hours of preservation observed in the present study was also comparable with the trends reported by Vyawanare et.al (1989) and Azawi et.al (1990).

In the present study, a highly significant difference was observed between the extenders in extra cellular leakage of AKP, ACP and LDH enzymes during preservation of Punganur bulls semen (Table-I) However, Dhami and Kodagali (1988) reported the effect of extenders on leakage of AKP to be highly significant, but on the leakage of ACP to be non significant in Surti buffalo bull spermatozoa. A significantly higher higher leakage of all the three enzymes viz., AKP, ACP and LDH noticed in Punganur bull semen preserved with EYC extender when compared to Tris extender which could be ascribed to the maximum protective action of Tris to sperm membrame against cellular injury compared to EYC extender. This observation was found to be in agreement with the reports of Vyawanare et al (1989) and Dhami and Sahni (1994). The release of various enzymes from spermatozoa may possibly result from membrane damage due to dilution as reported by Vyawanare et.al (1989).

In this study, a highly significant was noticed between bulls in the leakage of AKP and ACP but non significant difference was observed for LDH activity. This observation was found to be in agreement with the findings of Chanuhan and Srivastava (1973) who reported that phosphatases showed a highly significant difference between buffalo bulls and a non significant variation in respect to LDH leakage. A highly significant difference was also observed between storage times in leakage of AKP. ACP and LDH during preservation of Punganur bull semen in the present investigation. The leakage of all three enzymes increased with the increase in storage time in both the extenders. This is in agreement with the findings of Azawi et.al (1990) who reported an increased activity of AKP.ACP and LDH enzymes with the increase in the preservation time in Holstein Friesian bull semen.

Table –1

Enzyme levels (Mean ± SE) in Neat Semen and their Extra Cellular during preservation at Refrigeration temperature,

Neat Semen/ Extender	Enzyme		
	AKP (KAV/100ml)	ACP (KAU/100ml)	LDH (IU/Later)
Neat Semen	584.87± 11.22	241.47± 2.31	356.22 ± 9.45
TRIS			
0 hour	15.71 ± 2.08	8.92 ± 0.32	118.6 ± 5.25
24 hours	18.50 ± 0.29	10.41 ± 0.36	148.61 ± 1.99
48 hours	20.43 ± 2.00	11.97 ± 0.48	156.38 ± 2.07
Overall	18.21^{a} ± 1.22	10.44^{a} ± 0.35	141.20^{a} ± 2.98
EGG YOLK CITRATE			
0 hour	17.90 ± 2.11	11.25 ± 0.39	133.34 ± 4.56
24 hours	21.18 ± 2.13	12.52 ± 0.32	155.83 ± 1.58
48 hours	25.40 ± 1.78	13.74 ± 0.40	165.00 ± 1.42
Overall	21.49^{b} ± 1.25	12.26^{b} ± 0.34	131.38^{b} ± 2.46
AVERAGE (Tris and Egg Yolk Citrate)			
0 hours	16.80^{a} ± 1.49	10.09^{a}± 0.32	125.97^{a}± 3.68
24 hours	19.84^{b}± 0:17	11.46^{b}± 0.30	152.22^{b}± 1.40
48 hours	22.92^{c}± 1.42	12.85^{c}± 0.34	160.69^{c}± 1.44

Mean bearing different superscripts differ significantly (P<0.05)

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Effect of Thawing Rates on post Thaw Motility,Livability Acrosomal Integrity and Enzyme in HF Bull Semen

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ABSTRACT

Three thawing rates i.e 37°C/30 seconds, 45°C/15 seconds and 60°C/10 seconds were used for thawing of frozen HF bull semen diluted in Tris with and without sugar additives. Post thaw motility (PTM), Post thaw livability (PTL) Post thaw percent intact acrosomes (PIA) and leakage of enzymes i.e GOT, GPT< ACP and AIP were studied. The results revealed that the thawing at 45°C/15 seconds was better than two rates i.e 37°C/30 seconds and 60°C/10 seconds in terms of recovery of PTM, PTL and post thaw PIA. The addition of sugar in the diluting medium improved the post thaw semen quality with higher recovery and lower enzyme leakage.

Thawing of frozen bull spermatozoa at different temperature at varying duration has been studied with varied success. The extent of damage sustained by spermatozoa at the different thawing rates also varies. In earlier studies only one or two parameters has been taken as benchmark for declaring the results of frozen semen after thawing, post thaw motility being most widely studied parameter. However for better assessment of post thaw semen quality and assessment of damage to spermatozoa a number of parameters need to be studied. Therefore in the present study attempt has been made to adjudge the best thawing rate based on post thaw motility (PTM), post thaw livability (PTL), post thaw intact acrosome (PIA) and leakage of enzymes i.e GOT, GPT GPF Acid phosphatase (ACP) and Alkaline phosphatase (AIP)

MATERIALS AND METHODS

Semen ejaculates were collected in Artificial Vagina from 4 HF bulls maintained in the Germ Plasm Centre of Animal Reproduction Division. Fresh semen was evaluated for its physical characteristics and diluted in Tris dilutor containing 10% egg yolk and 6% glycerol. One part of diluted semen was added with 1% fructose as additive while the second part was used as control. (without sugar). The split semen samples were frozen in LN_2. After 24 hours the straws were thawed in water at three different rates of temperature and duration i.e 37°C for 30 seconds, 45°C for 15 seconds and 60°C for 10 seconds. The post-thew semen samples were evaluated for motility, livability and Acrosomal integrity (Watson 1975). Leakage of enzymes such as GOT acid and alkaline phosphatases were estimated in post thaw bull semen. (Reitman and Frankel, 1957), The data were analysed as per Snedecor and Cochran (1967).

RESULTS AND DISCUSSIONS

In the present study The results indicated (Table) that thawing of bull semen at 45°C for 15 seconds was better than two other rates at 37°C for 30 seconds and 60°C for 10 seconds in terms of recovery of highest PTM (48.75 ± 2.29 %) highest PTL (58.91 ± 1.5 %) and the highest PIA (62.00±1.4 %) followed by thawing at 37°C for 30 seconds in first combination of dilutor with sugar additive, similar pattern was observed amongst the three thawing rates in dilutors without sugar. However the values obtained for PTM, PTL and PIA were lower than the first combination of dilutor. The present results are in the similar pattern with those reported by Rodriguez et.al., (1975), and Robbin et al (1976).and who reported higher recovery after faster thawing at higher temperature than slower thawing at lower temperature. In the present study PTM, and PIA were recorded higher in the dilutor with sugar additive than in the dilutor without sugar additive. In the present study thawing rates exerted significant effect (P∠0.01) on the values of PTM, PTL and PIA whereas effect of dilutor combination was significant in case of PTM and PIA (P∠0.01) only and non significant in case of PTL,

In the present study the leakage of GOT, GPT was found to be significant (P∠0.05) due to the thawing rates. The addition of sugar (1% fructose) in the dilutor reduced the leakage of GOT and GPT in post-thaw HF semen which was statistically significant (P ∠0.01). The present results did not support the observation made by Pace and Graham (1971) who observed that thawing temperature had little effect on GOT release. However Tuli et. al (1982) ; Jani et.al (1983) observed significant increase in GOT release due to freezing. In the present study the highest leakage of GOT and GPT was recorded after thawing at 37°C for 30 seconds than thawing at 45°C for 15 seconds and at 60°C for 10 seconds.The results indicated that duration of exposure of spermatozoa to a certain temperature were more deterimental than the temperature itself.

In the case of ACP and AIP, the leakage of those two enzymes increased at 45°Cas compared to 37°C but it come down again at almost similar level at 60°C (Table.1). So the effect of thawing rates was non significant statistically on the leakage of ACP and AIP. The value of these two enzymes moved in a narrow range. The results of the present study differed with those reported by Pangaonkar et. al (1988). The difference might be due to variation in breed, freezing technique and dilutor combination etc.,

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Table.1 Effect of thawing rates on post-thaw physical and enzymatic attributes in HF bull semen.

	With sugar Additives (n=72)			Without sugar Additives (n=72)		
	TR-I	TR-II	TR-III	TR-I	TR-II	TR-III
PHYSICAL ATTRICUTES						
Post-thaw motility	41.75	48.75	37.50	35.41	41.41	30.25
	±2.37	±2.29	±2.35	±2.17	±2.14	±1.82
Post-thaw livability	51.58	58.91	46.41	49.16	56.16	44.58
	±2.13	±1.57	±1.67	±2.07	±1.63	±1.50
Post-thaw acrosonal	58.00	62.00	53.16	54.50	58.41	50.41
Integrity	±1.46	±1.44	±1.33	±1.38	±1.26	±1.10
Enzymatic Attributes						
Got	13.36	11.81	10.83	16.68	14.35	12.40
(u Mole/Litre)	±1.47	±1.43	±1.51	±1.44	±1.37	±1.34
GPT	7.99	7.46	6.89	10.16	9.26	8.35
(μ Mole/Litre)	±0.56	±0.59	±.0.53	±0.51	±0.55	±0.53
Acid Phosphates	41.11	37.19	41.85	41.16	37.39	41.88
(KA Units)	±2.65	±3.50	±3.86	±3.83	±3.54	±3.84
Alkaline Phosphates	30.72	36.68	30.96	31.03	36.82	30.42
(KA Units)	±3.59	±4.03	±3.39	±3.62	±4.05	±3.43

n =Number of observation TR-I=37^{O}C 0.30 seconds TR-II=45^{O}C/15 seconds TR-III=60^{O}C /10 seconds

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Spermatozoal Response to Hypo-osmotic Swelling and Freezability Tests after Testicular Degeneration in bucks

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ABSTRACT

Tellicherry bucks aged 3years were subjected to scrotal insulation with scrotal bag. The semen collected were subjected to HOST and freezability test. HOST positive, HOST negative and different types of HOST positive sperms were recorded and analysed statistically. The semen was unfit for cryopreservation from 2nd to 7th week and post-thaw live sperm count was low during first and 8th to 11th week post testicular degeneration. From 8th to 11th week though the semen was fit for freezing the post-thaw revival was poor.

Hypo-osmotic swelling test (HOST) is an endosmosis negative test that determines changes in the sperm membrane. The present investigation was taken up to assess the functional status and freezing capacity of spermatozoa after testicular degeneration.

MATERIALS AND METHODS

Six tellichery bucks aged 3 years were subjected to scortal insulation with scrotal bag for 48 hrs to induce testicular degeneration. Semen collection was made with the help of an artificial vagina. The mean data of two collections of six bucks taken in a week for 3 pre and 13 post-treatment weeks were grouped and analysed statistically.

HOS test was performed by combining 0.1 ml of ejaculate with1.0 ml of hypo-osmotic solution prepared by mixing 7.35g sodium citrate $2H_2O$ and 134.51g fructose in 1 litre of distilled water. The mixture was incubated for 60 minutes at 37 ^{o} C. Drop of the solution taken on microscopic slide were observed under high power and the percentage of spermatozoa with tail changes typical of HOS reaction was determined. Depending upon the intensity of coility and swelling of the tail, the spermatozoa were catagorised as type 1,2 and 3

Semen collected before and after testicular degeneration were subjected to test for freezability as per the method suggested to Sivaselvam (1992) for freezing of buck semen.

RESULTS AND DISCUSSIONS:

The percentageof sperm positive for HOST was 87.20 ± 0.38 in pre

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treatment control bucks. It reduced significantly to 50.13 ± 4.41 by 1st week and reached 11.09 ± 4.05 percent by 3rd week after testicular degeneration. Then by 12th week the value reached near normal level. (Table) there was corresponding increase and decline in the percentage of HOST negative sperm. The response of buck to HOST was similar to that reported for bovine (Drevius 1972) and human (Jayandran et al 1984).Furthur heat treatment of semen reduced the HOST positive sperm in human (Jayandran et al 1984) in canine (Kumi-Diaka, 1993) and in bulls Sivaramalingam, 1994)

The different types of response of sperms to HOST were categorised as type 1, 2 and 3 Drevius (1972) speculated that the type of swelling appears to be dependent on the factor of cellular water up take per unit time. Its level in pre-treatment control groups were 62.42 ± 0.38, 23.22 ± 0.28 and 1.57 ± 0.31 percent respectively. The incidence of type 1 and 2 declined significantly from 1st week and reached the lowest level of 6.30 ± 2.04 and 2.72 ± 1.18 percent on 3rd week. From 4 week their incidence increased and reached near normal level by 12th week.

The incidence of type 3 increased significantly to 8.47 ± 1.47 percent on 1st week then remained above normal level from 2nd to 11th week and reached close to pre-treatment control valueby12thweek.

Freezability of semen: The percentage of live sperms before freezing in the pre-treatment control was 92.83 ± 4.54. It significantly declined to 59.82 ± 5.08 percent by 1st week after testicular degeneration. During the subsequent period up to 7th week the live sperm count was very low and the semen was unfit for freezing (Table)

The freezability of spermatozoa was assessed by estimating the percentage of post-thaw live sperm. There was a sudden drop from pre treatment control value of 63.17 ± 0.44 percent to 43.85 ± 3.63 percent on 1st week post testicular degeneration. From 2nd to 7th week semen was unfit for freezing (table). From 8th week though the live sperm count increased and semen was fit for freezing until 11th week the post thaw revival was poor. This suggest that spermatozoa after testicular degeneration are more sensitive to freezing, since thermal insult appeared to become evident in an additive fashion as semen was further stressed by extension, freezing and incubation. Wettemann and Bazer (1985) observed that spermatozoa from rams with insulated scrotum was sensitive to freezing than from control rams.

Table

Relationship between HOS Test, prefreeze and post thaw Live spermatozoa

Treatment Period	Hos Positive Sperms %	Prefreeze Livesperms %	Post thaw Livesperms %
Pretreatmen Control	87.21 ± 0.38	92.83 ± 4.54	63.17 ± 0.44
Past treatment Period Weeks)			
1	50.13 ± 4.41 **	59.82 ± 5.08 **	43.85 ± 3.63 **
2	25.66 ± 3.75 **	15.27 ± 4.96 **	-
3	11.39 ± 4.05 **	12.48 ± 7.12 **	-
4	30.03± 1.47 **	11.36 ± 3.29 **	-
5	75.77 ± 1.59**	31.09±10.64**	-
6	61.58 ± 3.05**	23.55 ± 9.13 **	-
7	68.03 ± 2.21**	6.50 ± 7.62 **	-
8	72.17 ± 1.82 **	57.21 ± 4.47**	18.09 ± 1.94 **
9	69.50 ± 1.68 **	62.28 ± 4.90 **	28.24 ± 4.64 **
10	78.70 ± 1.35 **	86.11 ± 0.46^{ns}	53.84 ± 1.32 *
11	83.86 ± 0.78 *		
12	85.98 ± 0.68^{ns}	87.49± 0.37^{ns}	61.70 ± 0.83^{ns}
13	86.59 ± 0.61^{ns}	88.44 ± 0.72^{ns}	62.84 ± 0.95 ^{ns}

Post treatment values compared with pretreatment value in each column

** Highly significant ; * significant ; Ns -not significant

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In-Vitro Capacitation of Caprine sperm

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ABSTRACT

Goat epididymal and ejaculated spermatozoa were incubated in TCM-199 enriched with D-glucose. Calcium lactate, sodium pyruvate and goat serum (method 1) and modified Kreb's Ringer bicarbonate buffers containing pyruvate and lactate (method 2) Incubation and ejaculated semen was 3 and 4 hours respectively. Motility (whiplash motion of sperm tail) and visulization of acrosome reaction were used to assess sperm capacitation. Capacitation rate in terms of percent motility and acrosome reaction was higher in method 2 Difference in capacitation rate of cjaculated and epididymal semen in both groups were non significant (P>0.05).

Mammalian spermatozoa require to go for physiological changes (capacitiation) before they are able to penetratethe eggs. For in-vitro fertilization and production of embryos under laboratory conditions. a need for sperm capacitation under in-vitro conditions arises. A series of experiments were designed to evaluate different techniques and effectiveness of additives for inducing capaciation and acrosome reaction in ejaculated and epididynal caprine spermatozoa.

• Principal Scientist - Animal Reproduction

MATERIAL AND METHOD

Source and Recovery of Semen. Semen from intact bucks was collected using standard procedure. A female doe preferably in oestrus was used as dummy. For epididymal semen, testes from local abattoir were collected and processed within 1-2hr of the slaughter. Cauda cpididymes were dissected from the testes and washed 2-3 times in PBS. The spermatozoa were collected by cutting the cauda epididymis longitudinally.

Capacitation of Ejaculated Semen: In method I, the semen immediately after collection was allowed to stand undiluted at 20°C for 4 hours. The semen was then washed 3 times with BSA saline. resuspended in 2 ml (2 X 10^8 spermatozoa/ml) of modified M.199(pH 7.8) enriched with D-glucose (312.5mg/100ml). Calcium lactate (112.5) mg/100ml) sodium pyruvate (12.5 mg/100ml), bovine serum albumin (500 mg/100ml) and 20% oestrus goat serum and allowed to stand for 40 minutes (pre-incubation) at 20°C Thereafter, the sperm suspension was further diluted with the same medium of pH7.4 a final concentration of 1 X 10^6 cells/ml Caffeine @ 4mg/10ml was added to the medium. Semen motility and acrosome reaction of different stages of processing were studied.

The procedure of Anand et al (1989) with minor modifications was used in method 2. Immediately after collection, the semen was diluted and washed three times with BSA saline (0.1% bovine serum albumin, 0.9% sodium chloride. pH adjusted to 7.2). The washed pellet of goat spermatozoa was suspended in capacitation medium (CM-modified Kreb's Ringer bicarbonate buffered medium) at concentration 1 X 10^7 spermatozoa/ml The suspension medium was then distributed in small culture tubes, each containing 2 ml sperm suspension and incubated in a CO_2 incubator maintained at 37°C temp. 9.5% R.H.and 5% CO_2 in air. The samples were incubated for 4 hr and examined for motility and acrosome reaction.

Capacitation of Epididymal Semen: Methods used for ajaculated semen were used for in-vitro capacitation of epididymal semen. The only difference was in incubation period which was reduced to 3 hr in case of epididymal semen.

Motility Measurement and Acrosome Staining: Motility (whiplash motion of sperm tall) and visualization of acrosome reaction were used to assess capaciation. Sperm motility was assessed by examining a uniform drop of semen under a coverslip on a warm stage at 37°C. An arbitrary scale of 0 to 100 was used to assess it.

For acrosome morphology, a thin smear of semen on a glass slide was dried in air and fixed in formaldehyde solution at 37°C for 30 min. The slide was washed in water and kept in Giemsa Stain for 3 hr at 42°C. The slides were washed under tap water, air dried and examined microscopically under oil immersion. Three sequential stages of acrosome reaction, viz., acrosome swelling, acrosome vesiculation and acrosome shedding were considered.

RESULTS AND DISCUSION

Method: 1 Sperm motility and percent acrosome reaction of both ejaculated and epididymal semen are shown in table 1. Sperm motility of ejaculated semen improved after washing in BSA saline. Pre-incubation in medium 199 for 40 min showed an adverse effect on sperm motility and was reduced to 38.52 from 53.34 % Addition of caffeine in the medium improved motility. Percentage of spermatozoa exhibiting acrosome reaction increased with semen age during different stages of processing. Percent motility and acrosome reaction of epididymal semen were found at a higher ebb in most of the semen processing stages as compared to ejaculated semen

Table: 1 Motility (M) and Acrosome Reaction (AR) at different stages of semenprocessing (Method.I).

S.No	Different stages of semen processing	Ejaculated Semen		Epididymal Semen	
		M (%)	AR (%)	M (%)	AR (%)
1.	Fresh (neat) semen	42.46	3.2	---	---
2.	Semen after 4 h storage at 20° C	43.72	52.6	48.61	49.2
3.	Semen after washing in B.S.A saline	53.34	54.7	52.78	52.8
4.	Semen after preincubation for 40 min	38.52	67.8	62.00	62.6
5.	Semen after adding caffeine	46.81	72.5	66.78	67.5

Method 2: Sperm motility and percent acrosome reaction of both ejaculated and epididymal semen are shown in table 2. The motility of ejaculated semen after dilution in B.S.A saline was 58%. Washing of semen with BSA saline improved motility (78%) There was decline (62.50%) in motility after 4 hr incubation. A spurt in percentage of spermatozoa showing acrosome reaction was found 4 hrs after incubation. Epididymal semen at different stages of processing showed lower values in respect of percent motility and acrosome reactions.

Table 2: Motility (M) and Acrosome Reaction (AR) at different stages of semen Processing (Method 2)

S.No	Different stages of semen processing	Ejaculated Semen		Epididymal Semen	
		M (%)	AR (%)	M (%)	AR (%)
1.	Fresh (neat) semen	38.54	5.4	------	------
2.	Semen after dilution in BSA saline	58.00	8.2	53.62	8.6
3.	Semen after washing in BSA saline	78.00	12.6	65.00	10.08
4.	Semen after suspention incapacitation Medium	80.00	18.3	72.38	16.2
5.	Semen after incubation in capacitation Medium	62.50	81.0	60.00	72.8

Abundance of spermatozoa with head to head agglutination was observed in fresh ejacutated and epididymal semen. Subsequently spermatozoa began to come out of rouleaux and increasing number of sperm cells had free movement (whiplash motion). Motility to the extent of 72 and 80 percent respectively of epiddymal and ejaculated semen was achieved in method.2 Percent motility of both epididymal and ejaculated semen using method I was however low. Percent of spermatozoa showing acrome reaction increased steadily in both the groups. Based on motility and acrosome reaction, method 2 used for sperm capacitation was preferred.

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Capacitation of Buffalo Bull spermatozoa *in –vitro**

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ABSTRACT

In-vitro capacitation of buffaloes bull spermatozoa could be achieved by addition of 100 µgm of heparin. However heparin dosage of 100µgm /ml of media gave better results

In-Vitro Fertilisation (I.V.F) has been successful in various farm animals like Cattle, Goat, Pig, Sheep and Buffal. Standardisation of the technique has been obtained in most of these species, however the knowledge in Buffalo I.V.F, technique is limited (Sharma et. al, 1994). Therefore the following study was carried to study the capacitation process of In-vitro.

MATERIAL AND METHODS

The buffalo bull semen was obtained from DCKL, Aarey, Mumbai. The semen was diluted with egg yolk citrate and transported to the laboratory on ice. At the laboratory, 2ml of the liquid semen was taken in falcon tube and centrifuged at 500g for 5 min to separate the dilutor. The supernatant was discarded and the sperm pellet was resuspended in 1ml Tissue Culture Media-199(TCM-199). This resuspended spermatozoa was then placed at the bottom of four tube (0.25ml each) and 1ml of TCM-199 was layered above this and incubated at 38°C in 5% CO_2 and 100% humidity atmosphere for 1 hour swim-up. Following this the top 250 µl from each tube was pooled together in a separate falcon tube and centrifuged at 500g for 2 min. The supernatant fallcon tube and centrifuged at 500g for2 min. The supernatent was discarded and the sperm pellet was resuspended in 1 ml of TCM-199. To this 1 ml of capacitation media (TCM-199 with either 10 µgm heparin.ml or 100 µgm heparin/ml was added and incubated for 15 min.at 38°c,5% CO_2 and 100 % humidity atmosphere. Six replicates each for both the heparin dose was carried out. Capacitation of the spermatozoa was then confirmed by Geimsa stain. A drop of the capacitised sperm suspension was taken on a slide and a smear was prepared after air drying the smear was fixed in 90% alcohol for 10 mins, followed by immersion in the Gelmsa stain for 4 hours. The slides were then observed under microscope for acrosome reaction (A.R.) 100 spermatozoa were counted on each slide and the acrosome reaction percentage for the two levels of heparin

* Part of M.V.Sc., Thesis submitted by the author to Konkan Krishi Vidyapeeth,Dapoli

were calculated. Acrosome Reaction Rate = Spermatozoa showing A.R. X 100 / Total number of sperm counted

RESULTS AND DISCUSSION

The acrosome reaction percentage observed for µgm heparin/ml was 9.80 % and for 100 µgm heparin/ml was 25.33 % Thus capacitation was achieved by both the dosage of heparin, but 100 µgm/ml gave better results in buffaloes. Since in this study capacitation was studied by observing acrosome reaction only, the results were compaired with the IVF rates obtained by other workers who have judged capacitation with the fertilisation rate of the oocytes, matured In-vitro. The observation was in accordance in Fukui et al., (1991) who observed that in-vitro fertilisation rate was improved with increased dosage of heparin upto 100 µgm/ml in bovines, While Ahluwalia and Mujumdar (1992) achieved a better result in buffalo IVF studies when they used 100 µgm heparin/ml (IVF rate was 25.17%) as compared to Totey et.al (1991) who achieved 22.2 % IVF rate on using 10 µfm heparin +5 mM caffine for capacitation of the buffalo bull spermatozoa.

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Inorganic Elements and Their Seasonal variations in Seminal Plasma of Patanwadi Rams

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ABSTRACT

Estimation of inorganic elements in seminal plasma of Patanwadi rams revealed that calcium concentration was significantly higher during breeding season whereas inorganic phosphorus and Magnesium content showed non-significant seasonal variation.

The biochemical estimation of seminal plasma has assumed a considerable importance for andrological assessment of rams and also for evolving suitable preservation techniques and semen extenders. The present study is aimed to know the study in seasonal variation in the concentration of inorganic elements i.e Calcium, Magnesium and Phosphorus.

MATERIALS AND METHODS

Six adult healthy breeding Patanwadi rams were used for the study. The weekly semen samples collected by artificial vagina during pre-breeding (Feb-March)., breeding (April to 2nd week of June) and post-breeding (3rd week of June to end of July) season were biochemically after collecting the seminal plasma by centrifugation. Calcium was estimated using sodium chloranilate method of Webster (1962). Inorganic phosphorus was determined as per the method described by Taussky and Shorr (1953). The magnesium content was determined using Titan Yellow Method described by Oser (1965). The data were statistically analysed as per Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The calcium concentration (mg %) averaged 8.75 ± 0.28 (Table). The reported findings are in confirmity with the findings of Rai et. al. (1976) who studied calcium levels in seminal plasma of different breeds. However, much lower calcium contents (5.65 ± 0.04 mg %) were recorded by Daudu et.al (1984) in Yankeesa rams.

The observed difference between seasons was found highly significant ($P<0.01$) Calcium content was recorded highest during breeding season and it significantly differed from pre-breeding and post-breeding seasons. The calcium content was almost similar during pre-breeding and post-breeding seasons.

The inorganic phosphorus (mg %) averaged 10.25 ± 0.21 (Table). These findings are in consonance with Varshney et. al. (1977) who reported the inorganic phosphorus content 10.59 ± 0.90 mg per 100 ml of seminal plasma in bucks

The observed difference between the seasons were found to be non-significant. The inorganic phosphorus content was almost constant in all seasons.

The Magnesium content (mg %) averaged 4.94 ± 0.10 in patanwadi rams (Table) The findings are nearer to the findings of Mann (1964). However, much lower magnesium content (2 mg %) was reported by White (1958). The observed difference between seasons was found non-significant indicating that the magnesium content of seminal plasma was not affected by season.

Average (Mean ± S.E) value of inorganic elements in Patanwadi ram semen during different seasons.

Constituents	Pre-breeding season	Breeding	Post-breeding	Overall mean
Calcium (mg %)	8.09 ± 0.42	9.35 ± 0.43	7.8 ± 0.40	8.75 ± 0.28
Inorganic Phosphorus (mg %)	10.72 ± 0.47	10.30 ± 0.31	9.72 ± 0.26	10.25 ± 0.21
Magnesium (mg %)	4.65 ± 0.25	5.05 ± 0.14	5.07 ± 0.08	4.94 ±0.10

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Preservation of Native Boar Semen

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ABSTRACT

The present study indicates that the native boars semen could be preserved successfully at 15°C beyond 48 hours with optimum percentage of motility in four diluents viz., 1. Glucose Potassium sodium tartrate sodium citrate dihydratt 2. Kiev I.3. Kiev II 4. Glucose – Glycine EDTA-sodium bicarbonate citrate.Where as preservation at 5°C have been found less favourable beyond 48 hours.

Many authors reported the preservation of boar semen using different diluents (Vijayakumaran & Iyer, 1980, Tamuli et al., 1986) in exotic boars, but the reports on preservation of native boar semen are scantly, Hence the present study was undertaken.

MATERIALS AND METHODS

The present study was carried out on five native boars aged 12 to 14 months maintained at A.I.C.R.P on pigs, C.V.Sc., Tirupati. A total of 50 collections, ten from each boar were collected to study the preservability of the spermatozoa at 5°C and 15° C in four diluents Glucose-Potassium-Sodium tartate-Sodium citrate dihydrate Edate (G.P.S.E) 1, Kiev I, Kiev II and Glucose-glycine, EDTA sodium bicarbonate citrate (G.G.E.B.C) the extenders were prepared and autociaved as per Murthy & Rao (1975). The semen samples having more than 90% of progressive motility were utilized to study the preservability at 5°C (t_1) and 15°C (t_9). The methods described by Snedecor and Cochran (1967) were adopted to analyse the date.

RESULTS AND DISCUSSION

The results are presented in Table. It was observed that the motility of boar spermatozoa was superior in all the diluents at 15°C than at the 5°C. Similar findings were reported by Murthy & Rao (1975) Kasuya and Kawbe (1980) Vijayakumaran & Iyer (1980) in exotic boars Venumanohara Rao (1989) in native boars.

The sperm motility was uniformly poor in all the diluents at 5°C and dropped to less than 40% after 60 hours of preservation. The progressive motility was more than 50% in all the diluents even after 48 hours at 15°C.

The preservation of native boar semen was good in G.P.S.E diluent at 5°C and 15°C followed by Kiev I, Kiev II and G.B.E.B.C which was in concurrence with reports of Tamuli et al., (1986) in exotic boar, Venumanohara Rao (1989) in native boars. The poor preservability of boar semen at 5°C when compared to

15°C might to due to change in solubility of certain components of the diluent and pH of the diluent at lower temperatures (Cole and Cuppes, 1968). The reasons for the better preservability in G.P.S.E diluent may be due to presence of EDTA, tartarate and citrate in it. Where as in the other three diluents did not contain tartarate and comprised of EDTA and citrate in common. Though citrate and EDTA are present in other diluents the citrate presents in the G.P.S.E. might have synergistic action in preventing the swelling of spermatozoa than either one of the ingredients alone. The percentage of motilityis low in Kiev.II diluent than Kiev I both at 5°C and 15°C. This might be due to presence of chlorideions in Kiev II diluent, which have the property of favouring the swelling of the spermatozoa (Tomar, 1970).

Table:1 Percentage of progressively motile spermatozoa in different diluents at different hours of preservation at 5°C and 15°C Temperature.

Hours of Preservation	Preservation at 5°C				Preservation at 15°C			
	Diluents				Diluents			
	GPSE	GGEBC	KIEV.I	KIEV.II	GPSE	GGEBC	KIEV.I	KIEV.II
12	83.40	70.80	77.90	67.10	85.20	72.70	77.90	74.70
24	74.95	61.60	67.20	58.10	79.48	65.20	68.10	67.70
36	67.60	50.80	58.60	48.00	71.10	56.00	62.90	60.30
48	58.30	42.30	48.70	39.80	64.40	48.40	54.20	50.00
60	48.80	35.90	39.80	33.40	56.70	42.80	44.10	42.80
72	38.70	27.70	34.80	24.20	48.90	35.90	36.70	43.20
84	26.10	17.00	25.30	16.70	40.30	26.70	26.90	29.20
96	16.00	6.70	16.50	5.90	31.50	15.70	17.90	19.60
Mean	51.73	39.03	46.10	36.65	59.69	45.43	48.59	48.44

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Recovery Percentage of Culturable Buffalo Oocytes From different sized ovarian Follicles

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Massive number of buffaloes are bought to urban areas each year and slaughtered after compleation of their lactation leading to direct loss of valuable germ plasm (Sharma et. al., 1994). The following study was taken up with the objective of assessing the quality of oocytes obtained from different sized follicle.

MATERIALS AND METHOD

Buffalo ovaries of different breed used for the present study were obtained immediately after slaughter from abbatoir. Ovaries were transported to the laboratory in a flask containing 0.9% saline at room temperature. The follicles on the ovaries were measured using vernier caliper and based on their diameter were divided into two groups viiz., Group.A 3-5 mm and Group B>5 mm. The oocytes were aspirated along with the follicular fluid with the help of tuberculine syringe fitted with 23 guage needle. The oocytes collected from these follicles were then classified into GOOD-oocytes with complete thick compact cumulus and ooplasm appearing evenly grannular completely filing the zona; FAIR oocytes with partial/incomplete investment and ooplasm showing uneven grannulation of black bodies, but filling the zona; POOR-oocytes with expanded or absent cumulus and ooplasm shrunken or vacuolated. The oocytes aspirated were placed in a 60mm petri dish along with the follicular fluid, and observed under steriozoom microscope for grading and selection of oocytes.

RESULTS AND DISCUSSION

Out of 583 follicles aspirated, the overall recovery percentage was 52.31 % and for the two sized follicles it was 51.05% (n=169) and 53.96 % (n=136) respectively. The findings are in agreement to that of Liebfried and First (1979) who observed 50% recovery from follicles of 1-3 mm and >3 mm size. In this study, out of 169 cocytes recovered from follicle size. 3-5 mm, 39.05 % were GOOD; 39-05 % were FAIR and 19.52 % were POOR. Whereas out of 130 oocytes recovered from follicle size > 5mm, 40.44 % were GOOD ; 47.05 % were FAIR and 12.5 % were POOR quality oocytes. It was observed that the percentage of fair quality oocytes recovered from follicles of larger diameter are higher as compared to smaller diameter follicles collicles, this is similar to the observation recorded by Selvaraj et.al (1992). In the present study, out of the total 305 oocytes collected, the overall percentage of

GOOD FAIR and POOR quality oocytes were 39.67 % 42.62 % and 16.42 % respectively. The Overall percentage of Fair quality of oocytes was more than GOOD quality oocytes which is similar to that observed by Giri (1992) Longergan et.al (1991) and Carolana et.al (1994) From the above study it is concluded that overall recovery percentage of oocytes from various sized follicles is 50 percent and the percentage of culturable oocytes from group A and group B follicles were 70.50 % and 87.50 % respectively indicating that higher percentage of culturable oocytes can be achieved from follicles of large diameter

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Relationship between sperm percentage and progressive motility in cattle and buffalo Semen.

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Total number of live spermatozoa is usually greater than the total motile spermatozoa as all the live sperms are not always motile. It is useful to estimate the percentage of live spermatozoa in a semen sample, in addition to progressive motility, since some live non-motile spermatozoa become motile sometimes after storage (Tomar et al., 1969) However according to Salisbury et.,el (1978) live dead staining is not superior to motility estimates in predicting the survival of sperm cells through the freeze-thaw process.

Since these two are the related criteria to judge the quality of semen to be used for insemination, the present study was aimed to know the exact relationship between them.

MATERIALS AND METHODS

Frozen semen straws from 6 Jesey, 3 cross-bred (Jersey X Red Sindhi) and 4 Murrah buffalo bulls of known fertility were used in this study. These bulls were stationed at intensive Livestock Improvement Programme. Semen Processing Laboratory. Palampur, Himachal Pradesh and were being used routinely in the A.I.programme of the State Government Semen collection, processing and freezing was done as per routine procedure and Tris extender was used for dilution of semen.

For this study 10 different batches, each comprising of 9 straws per batch were obtained from these bulls (90 straws from each species/breed). These straws were thawed in a constant temperature water bath at 40°C for 14 seconds Immediately after thawing, semen from one straws of each batch was examined for progressive motility and live sperm percentage The remaining 8 straws from each batch were divided into two sets. One set of straws was placed in a thermos flask containing water at 37°C and was incubated for 4 hours. Another set of straws was wrapped in cotton and stored at room temperature. Semen quality from one straw was again evaluated at hourly interval. Live sperm percentage of freshly thawed and post incubation semen was compared with corresponding figures of progressive motility. Correlation and regression estimates were carried out according to Snedecor and Cochram. (1967).

RESULTS AND DISCUSSION

There was a progressive fall in the sperm motility following decline in live sperm percentage. The observed difference within a breed between treatments (water and cotton incubation) were not significant. Since the estimates of regression coefficient did not differ between treatment, the date, of two treatments within a breed/species were pooled. It is apparent from Table that the live percentage was significantly correlated (P< 0.01) with the progressive motility within jersey (r=0.96 ± 0.02, t =48, n=100), cross-bred (r=0.96 ± 0.02 t=48, n=100) and buffalo semen (t=0.93 ± 0.03, r=31, n=100. The regression of progressive motility on live sperm percentage was 0.91 ± 0.002 (5=45.5 n=100) in jersey, 0.96 ± 0.02 (t=48, n=100) in cross-bred and 0.98 ± 0.03 (t=32.6. n=100) in buffalo bull semen. The difference between species was not significant.

Thus , for every 10% decrease in post thaw live sperm percentage, the progressive motility declined by 9.1% in Jersey, 9.6% in cross-bred and 9.8% in buffalo bull semen.

Table:1 **Relationship between live sperm percentage and progressive Motility in cattle and buffalo.**

S.NO	Post-thaw incubation	Correlation Coefficient	Regression estimate	Regression equation
1	Jersey semen			
	In water	0.96 ± 0.03	0.97 ± 0.03	-10.70 + 0.97x
	In cotton	0.97 ± 0.03	0.86 ± 0.03	- 3.59 + 0.86x
	Pooled	0.96 ± 0.02	0.91 ± 0.02	- 7.23 + 0.92x
2	Cross-bred semen			
	In water	0.96 ± 0.04	0.99 ± 0.04	-12.09 + 0.99x
	In cotton	0.96 ± 0.03	0.93 ± 0.03	- 7.88 + 0.93x
	Pooled	0.96 ± 0.02	0.96 ± 0.02	-10.01 + 0.96x
3	Buffalo semen			
	In water	0.93 ± 0.05	1.0 ± 0.05	-18.56 + 1.00x
	In cotton	0.93 ± 0.02	0.97 ± 0.05	- 1.55 + 0.97x
	Pooled	0.93 ± 0.03	0.98 ± 0.03	-17.33 + 0.99x

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Seminoma in a Cryptorchid Bull

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Primary testicular tumors although common in dogs, are rare in bulls. Isolated cases of testicular turnors viz., Sertoli cell tumor, Interstitial cell tumor, Seminoma and Lipoma were recorded in India by Bhagwat et.al (1972) and Sharma et. al (1978). The present study reports a rare case of Seminoma in a Cryptorchild bull.

A Hallikar bull aged four years with the history of gradual enlargement of testis since six months, was brought to the Veterinary Hospital, C.K.Palli, Ananthapur for treatment .On examination, the bull was found to be a unilateral cryptorchid and the right testis in the scrotum enlarged more than double in size. No fluid or pus escaped on exploratory puncture. Surgical removal was undertaken suspecting testicular neoplasia or hypertrophy.

On gross examination, grey, lobulated tumor mass was bulging out from the testis and the tumor mass was separated from healthy tissue. Histological sections prepared showed highly vascular stroma with hemorrhages. The neoplastic cells were arranged as islands, separated by connective tissue. The neoplastic cells were large, rounded with granular cytoplasm. The nucleus was hyperechromatic. One or two nucleoli and few mitotic figures were present. On the basis of morphology, histological character and location of growth the tumor was diagnosed as seminoma. Bhagwat et.al (1972) reported a case of bovine Seminoma due to severing of spermatic cords by open method of castration. In the present case cryptorchidism might be the predisposing factor which gains support from the observation of Sastry (1979)

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A Preliminary study on the use of abdominal cannula for frequent viewing of abdominal organs in Caprines

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The information available on various reproductive events like, ovulation rate, ovulation time, sequence of follicular growth, development of corpus luteum etc., in goats and to explain the causes of infertility in these animals is primarily based on either laparotomy findings or examination of the abbatoir material. With the advent of laparoscopy the viewing of internal organs has become simple. However, for frequent laparoscopic observations, repeated punctures are to be made in the abdominal wall subjecting the animals to stress at each time. The implantation of abdominal cannual for repeated laparoscopic examination may be a better alternative.

The present study was undertaken to develop an abdominal cannula for goats. The cannula was devised from the barrel and piston of 1 10 ml plastic syringe, measuring 5.5 cm in length and 1.2 cm internal diameter (fig.1). The cannula was implanted at midventral aspect (Gr.I, 6 animals) and right paralumbar fossa (Gr.II, 6 animals) on Surti or Marwari adult femals goats and retained for a period of 24 days.

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During the period of study animals were regularly monitored for the retention of cannula, tissue reaction and laparoscopic viewing of abdominal organs. The animals of Group I were sedated using xylaxine 0.5 ml i.m. and restrained in dorsal recumbency. A skin incision, 2-3 cm was made, 4-5 cm crannial to undder, lateral to linea alba, and modial to corresponding milk vein under local infiltration of xylocaine. It was deepened, incising the muscles and peritoneum. The sterlized cannula was gently introduced through the vent and retained in position with the help of nylon sutures. Group II animals were secured in the left lateral recumbency after sedation. The abdominal cannula was implanted in the right para lumber fossa under local infiltration of xylocaine using the same procedure as in animals of group-I. The abdominal cannula was retained in position and the wound was closed.

In the animals of group I, there was accumulation of exudate in the cannula. The exudate was initially watery in consistency and from day 4 onward the fluid accumulation decreased and its consistency became thick and viscous. From day 15 post implantation there was no accumulation of exudate.

The animals of group II had no accumulation of fluid in the cannula. Slight swelling at the implantation site along with oozing of watery discharge was observed from the ventral margin of skin wound for first three days. The skin opposing the wings of cannulae did not show any tissue reaction. The visualization of internal organs revealed no injury to internal organs or development of abhesions. In the animals of group II the viewing of internal organs was better in standing position. Wani (1982) used various approaches for laparoscopy and advocated midventral approach to be the ideal one. However, in the present study the viewing of internal organs through right para lumber fossa was more convenient. Mutiga and Baker (1984) also suggested the dorsal area of para lumber fossa to be ideal for laparoscopy particularly for embryo transfer in standing ewes. In the present study, the animals of both the groups developed a membranous structure at internal opening of cannula which had to be penetreated for getting clear view of internal organs, Dougheity (1981) also reported the formation of peritoneal like deposits on the inner surface of plastic windows in the abdominal wall used for observation of ovarian activity in cattle.

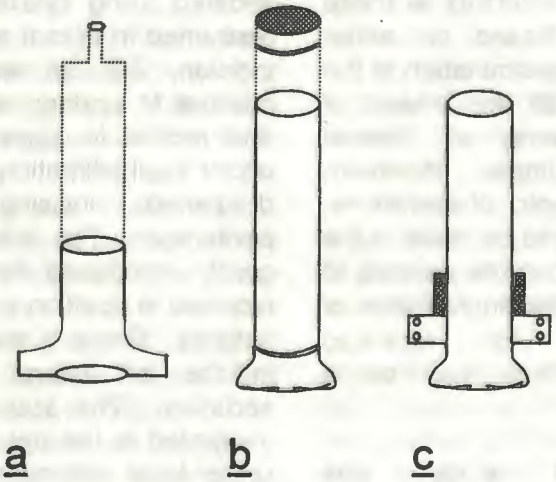

Fig.1 : Parts of plastic syringe to be used for preparing abdominal cannula

a. Barrel of Syringe (broken line –––––) indicate the portion to be discarded;
b. Piston of syringe with lid (I) at distal end broken line (–––––) portion to be discarded.
c. Developed abdominal cannula with portion of barrel (ii) fixed to distal end of piston.

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Effect of Prostaglandin F2 alpha on induction of Parturition in she camel (Camelus dromedarius)

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Ever since the initial reports of the use of coticosteroids commonly used in large farm animals for the induction of parturition (Adams, 1969,) and corticosteroids and prostaglandins in combination (Morrow, 1986) several successful reports have appeared in the literature. Perusal of literature did not show any report on this aspect in she camel in this country.

On 10/03/1996 a she camel with a history of dystocia in the previous parturition was presented with a report of exceeding normal gestation period 389.3 ± 0.08 days. (Khanna et. al; 1990) by 24 days. Clinical examination revealed staggering gait and hind quarters were unable to bear weight. Gynaeco-clinical examination revealed foetus in posterior presentation with no foetal movements and a feeble pulsation of uterine artery. The external 0S was closed and no labour pains were observed.

Initially a dose of PGF2 alpha 30 mg (Lutalyse, Unichem Laboratories ltd. Bombay) was administered intramuscularly. Supportive treatment with parenteral fluids and antibiotics was followed for the next three consecutive days. Vaginal examination after 24 h of treatment revealed no conspicuous change in the condition of external os. At 48 h in the cervix was partially open admitting one finger while at 72 h it was sufficiently dilated to allow the hand inside the uterus through the cervix and with manual manoevering a male dead foetus was recovered in posterior presentation.

Prior to attempting retraction the uterus was well lubricated with bland oil. Following delivery of foetus Teramycin bolus 500 mgx 10 (Pfizer limited), were placed inside the uterus to check the infection. However, The animal died on the fourth day. Induction of parturition with corticosteroids to be effective requires a live foetus of at least 7 months old (Vandeplassche et. al 1974) however in the present study the foetus was normal gestation and was delivered dead.

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Mummification in a Churi

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Yak (Bos-gunniens) are the integral part of agriculture in tribal areas of Himachal Pradesh. For the improvement of it's milk production,cross-breeding with Jersey germ plasm is becoming popular. The female cross between yak and cattle is called a Churi.

A Churi aged 7 years was presented in veterinary hospital Dharwas in Pangi valley of tribal belt of Himachal Pradesh with the history of prolonged gestation. The animal was brought by the owner. for examination, when no signs of parturition were observed in an earlier confirmed pregnant churi even after one month of expected date. Per rectal examination revealed a solid contracted mass inside the uterus. The Uterus was palpable just in front of pelvic brim ,lacked fluid and was closely applied to the fetal mass. There were no cotyledons and ovaries were not palpable vaginal examination disclosed a closed cervix. The case was diagnosed as mummiification.

Due to non availability of prostaglandin in this remotest parts of the state, it was decided to perform laparo-hysterotomy,. Laparotomy was resorted to under xylazine sedation and local anaesthesia. The thick, tightly contracted uterus was exteriorised and about 3 months old fetal mummy was taken out through left paramedian incision.

Thick chocolate coloured semi viscous material was present in between uterus and fetus. Closure of uterine incision was preceded by vigorous flusing of the uterine cavity with antibiotic solution. The surgical wound was closed in routine manner. Following 5 days post-operative care and treatment animal was discharged with uneventful recovery.

Bovine mummified fetuses may be found at routine clinical pregnancy examination or may be discovered when no signs of approaching parturition are seen in confirmed pregnant animals, (Arthur et., el.,1989). This case was also diagnosed when the earlier confirmed pregnant Churi failed to calf after completion of term. For the treatment of cows pregnant with mummified fetuses two methods are recommended. The first is to initiate the parturition mechanism and second is to perform hysterotomy (Arthur et. al 1989). Is the present case due to non availability of prostaglandin, second method was adopted with favourable results.

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Biochemical constituents of Foetal fluid During late Gestation in Non descript Bitches

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Metabolism of foetus during its development in the uterus influences the biochemical constituents of the foetal fluid. These constituents varies with the age of foetus during gestation. (Baetz et.al., 1976). The dynamic changes in the biochemical constituents of foetal fluid has been studied in detail in cattle (Sloss and Duffy, 1980), in mares (Williams et. al., (1993), in sows (Goldstein et al., 1980) and in sheep and goat (Wintour et. al ., 1993) Perusal of literature indicated that no work was conducted to study the biochemical constituents of pregnant bitches. Hence the present study was formulated to investigate the certain biochemical constituents of foetal fluid during late gestation in non descript bitches.

Ten, non descript bitches between the age of 2-3 years were selected and bred with fertile dog and their pregnancy was confirmed by radiography. During 50-60 days of gestation, caesearean section was performed under general anaesthesia in all manner without damaging the foetal sacs using 24 gauge needle fitted with 2 ml syringe. The collected amniotic and allontoic fluids from all foetus (5-7 number) of each bitch pooled separately and stored – 20°C until biochemical analysis. Enough care was taken to collect the fluids without blood contamination. The amniotic and allontoic fluids were analyzed for urea nitrogen, creatinine, total protein, albumin, sodium, potassium, calcium and phosphorous by using BTS-320 Photometer and commercially available kits, (Stangen immunodiagnostics). Composition of amniotic and allontoic fluid was compared by student 't' test. (Snedecor and Cochran, 1967).

The estimated values (mean ± S.E.) of urea nitrogen (mg %) 10.42 ± 0.2674 and 34.34 ± 1.004, creatinine (mg %) 2.01 ± 0.0979 and 6.27 ± 0.1505. Total protein (g %) 0.31 ± 0.283 and 0.50 ± 0.0084, Albumin (g%) 0.31 ± 0.283 and 0.50 ± 0.0084. Sodium (mmol/L 164.50 ± 1.4558 and 283 ± 0.8406, potassium (mmol/L) 5.56 ± 0.1278 and 7.08 ± 0.0827, Calcium (mg %) 4.54 ± 0.1194 and 28.70 ± 0.4232 and phosphorous

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(mg %) 8.47 ± 0.1193 and 13.44 ± 0.5815 for amniotic and allontoic fluid, respectively. The values of allantoic fluid significantly (P< 0.01) higher than that of amniotic fluid.A moderate variation in colour of both fluids was observed between bitches, however the allontoic fluid was straw yellow and amniotic fluid was clear to opaque in all samples

Based on the results from this investigation, it can be concluded that the biochemical values of bitch allontoic fluid are statistically higher than that of amniotic fluid in late gestation. Efforts to collect the foetal fluid at different stage of gestation and the knowledge of the biochemical constituents of the collected fluid will help to assess the normalcy of the foetal development .. More over it supports the existing knowledge of foetal fluid production (Roberts, 1971).

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Reproductive Performance of Jaffrabadi buffaloes in Saurashtra

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Jaffrabadi buffaloes constitute 23.42 per cent of total 53.10 lakh buffalo population in Gujarat (Anonymous 1992) and are well known for their milk production ability (2,000 liters/lactation) and high fat (8.5 %) content with larger fat globules. They are uncertain breeders and have a long intercalving period (Ranjhan and Pathak. 1993). Basic information on some of the reproductive traits of these buffaloes is necessary to improve the breed with a view to economise the buffalo husbandry practices in Saurashtra.

MATERIALS AND METHODS

Jaffrabadi buffaloes (n=189) of Cattle Breeding Farm, Gujaraj Agricultural University. Junagadh formed the base for this study. Data from 1987 onwards for a period of ten years were analyzed on Post Partum estrus, Service Period, incidence of reproductive disorders in females number of services per conception and fertility percentage of bulls based on confirmed pregnancy. The data were statistically analyzed Snedecor and Cochran 1967).

RESULT AND DISCUSSION

Post Partum Oestrus: The day of oestrus symptoms following parturition varied from 79.66 ± 11.48 in the year 1994 to 136.95 ± 27.43 in the year 1990 the difference being non-significant (P> 0.05). The average post partum estrus in Jaffrabadi buffalo herd of this farm is 113.16 ± 8.74 days, in comparision to 134.9 ± 6.49 days in Murrah breed (Ray and Pandey, 1971) and 125.73 days in Surti breed (Rao et. al 1973) Saurashtra region which is the home tract of Jaffrabadi is prone to frequent droughts, consequently, scarce water and feed resources are the main constraints during these periods affecting buffalo reproduction.

Service Period: Average Service Period of Jaffrabadi herd was 137.96 ± 6.83 days ranging from 100.21 ± 13.91 days in 1987 to 100.87 ± 21.80 days in 1994. It is comparable with those of 68-218 days in Murrah and 193-236 days in Surti buffaloes (Nagarcenkar 1979).

Conception Rate: In Jaffrabadi buffaloes, average number of services per conception ranged from 1.14 ± 0.14 in 1993 to 1.61 ± 0.13 in 1989 with an overall average of 1.41 ± 0.07. The average number of services required per conception reported by different research workers in Indian buffaloes are 1.81 ± 0.02 in Mehsani (Dave and Parekh, 1976). 1.31 in Nagpuri (Kadu, 1978 and 1.90 in Murrah (Singh et.al 1979).

Reproductive Disorders : During the period under study with a total buffalo population of 315 at risk, the major incidence of disorders (based on percentage of total calvings) appeared to be genital prolaps (9.21 %) followed by metritis (6.98%) and retained placenta (6.03%). The incidence of abortion (2.45%) still birth (1.59%) and dystocia (1.27 %) were found to be minimum.

Breeding Performance: Jaffrabadi bulls are extensively used for breeding the buffalo herd on this farm. They are generally sluggish and need greater attention by the attendents for each service performed. The average services, required per conception varied from 1.25 in 1987 to 2.21 in 1988. Fertility percentage of bulls based on confirmed pregnancies ranged from 45.25 (1988) to 80.00 (1987), yearly differences being significant ($P<0.05$).

Thus overall reproductive performance in Jaffrabadi breed is comparable to the other Indian buffalo breeds and there is scope for further improvement by modifying/providing suitable managerial conditions.

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Study on the efficacy of progesterone substitution therapy in Repeat Breeding cows

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Embryonic mortality after breeding was considered to be the major cause for the reproductive failure. Sreenan and Disken (1985) observed that 40 percent of the fertilized ova were lost due to embryonic mortality. It has been inferred that the spontaneously occuring asynchrony between the embryo and meternal enviornment was the prime cause for the embryonic mortality. Maurer and Echternkamp 1982) Measures commonly practiced to achieve and maintain "Meternal synchrony" are administration of either progesterone or luteinizing hormone. Hence in the present study repeat breeding cows were subjected to progesterone therapy and the efficacy of the therapy is discussed.

MATERIAL AND METHODS

Fifty two parous cross bred cows with a history of normal calving and not conceiving subsequently, inspite of repeated insemination, were included in this study. The cows were subjected to a thorough gynocological examination to rule out any abnormality. After the examination these cows were inseminated with good quality frozen semen. Progesterone 500 mg (Hydroxy progesterone Hexonate – Tablets India) was administred to these cows on day 5 of the oestrous cycle and advised to bring the animal after 60 days for pregnancy verification.

RESULTS AND DISCUSSION

Out of the 52 cows administred with progesterone 13 cows were brought for insemination after 21 days. Thirty nine cows were brought for pregnancy diagnosis between day 60 to 90 and 28 cows were diagnosed as pregnant resulting in 53.84 % of first service conception.

Wilmut et.al (1986) indicated that early embroynic loss could occur through inadequate uterine environment. Endometrial secretion, essential for stimulating and mediating changes in conceptus growth and differentiation throughout early pregnancy was directed by the steroid environment generated by the ovary. Maurer and Echternkamp (1982) observed that a higher progesteone concentration and a lower ratio of estrodial to progesterone on day 3 and 6 was very conducive for the growth of the embryo.

Rosen and Struman (1989) observed that in repeat breeder cows parentral administration of progesterone in 5-10 days of oestrous cycle had improved the conseption rate significantly. Walten et. al (1990) observed that the parental administration of progesterone in cows resulted in increased embryo viability and conception rate. In embryo transfer technology progesterone treatment to receipient cows, during 1-5 days of oestrus cycle promoted the survival rate of trasnfered frozen embryos and improved pregnancy rate (Smyslova et al 1988; Geisert et. al 1991). Devanathan and Pattabiraman (1997) observed that the administration of the progesterone brought about a certain change in the uterine luminal sercretion.

It is concluded that the administration of progesterone to repeat breeding on day of 5 of the oestrous cycle following A.I increased conception rate.

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Pregnancy diagnosis in the sow by vaginal Biopsy Technique

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Dyck (1992) compared all the prevailing methods of pregnancy diagnosis in the sows and observed that vaginal biopsy technique had many advantages viz., high accuracy and low cost over other methods such as ultrasound and hormonal assay. Therefore, the present work was under take to evaluate vaginal biopsy technique for pregnancy diagnosis in sows.

The study was conducted at Government pig breeding Farm, Ranchi. Pregnancy diagnosis was made in 50 large white Yarkshire sows. Biopsy samples were taken from anterior vagine between 20-22 days post service. The biopsy instrument consisted of a 50cm. Long stainless steel tube of 5mm. diameter with cutting edge at one end was used

The sows were restrained properly in the stall. Biopsy samples were collected generally at the time of feeding. The vulva and perineal region was cleaned properly with soap and water and dried. The instrument was introduced into the vagina about 15-20 cm. deep with the cutting edge closed. The tip was pressed against the vaginal wall by tilting the cuter portion of the biopsy instrument to an angle of about 45^{0}. The inner rod was rotated so that the cutting edge was exposed and the rotation continued further 180^{0}. Subsequently the instrument was withdrawn. The piece of vaginal mucosa was transferred to a small vial containing 10 ml. of 10 percent buffered formal saline. Paraffin sections (6-8 micron) were cut and stained with H & E The slides were examined under high power magnification for counting the epithelial and cellular proliferation.

The characteristic feature observed during pregnancy was 2-3 parallal layers of vaginal epithelium with darkly stained nuclei. The pregnancy diagnosis was observed to be 96 per cent accurate as 48 sows furrowed. Diehl and Day (1973) also found an accuracy rate of 95.5 percent which is in agreement with the present findings.

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