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EFFECT OF FSH, LH AND ESTRADIOL – 17 β ON IN VITRO MATURATION OF BUFFALO OOCYTES

S.K. SACHAN, Y.P.S.DABAS and S.N. MAURYA

Department of Gynaecology and Obstetries,
College of Veterinary Science
G.B.Pant University of Agri. & Tech. Pantnagar – 263 145

ABSTRACT

Oocytes aspirated from the buffalo ovaries were transferred to one of the five combinations of maturation media viz., supplemented with 100 IU LH (Gr.1), 1 µg Estradiol (Gr.II), 1 µg FSH (Gr.III), combination of LH, FSH and Estradiol (Gr.IV) per ml and control (Gr.V) with no hormone supplementation and incubated for 24hrs in 5% CO_2 in air with 95% humidity at 39°C. TCM-199 supplemented with 10% FCS served as basic maturation medium. The oocyte maturation rate was significantly increased on supplementation of maturation medium with FSH and a combination of FSH, LH and estradiol. However, presence of LH alone or in the presence of FSH and Estradiol in maturation media resulted in lowering of in vitro maturation rate of buffalo oocyte.

In vitro maturation of oocytes is an integral part of the IVF system. Hormonal stimulation of oocytes during the course of in vitro maturation is of paramount importance in achieving nuclear and cytoplasmic maturation which is essential for preparation of oocytes for fertilization (Stubbings et. al.. 1988).

The present study was, therefore, undertaken with the objective to study the effect of FSH, LH and Estradiol-17 beta either alone or in combination on in vitro maturation of buffaloe oocytes

MATERIALS AND METHODS

Buffalo ovaries were collected from slaughter house and transported in Dulbeco Phosphate Buffered Saline (DPBS) added with penicillin (4000 units/litre) to the laboratory. The ovaries were washed 5 to 6 times with lukewarm DPBS followed by 60 % ethanol to remove the gross contamination. Fullicular fluid was aspirated from small to medium sized (2-6 mm) antral follicles and was carefully poured in sterile test tube and the search of oocytes was carried out under stereozoom microscope. The aspirated immature oocytes were serially washed 5 times in 50 µl drops of washing medium ((HEPES-240 mg, BSA fraction V-700 mg, TL-bass-100 ml, pH 7.3-7.4)

The basic maturation medium was prepared with TCM-199 (15.6 mg/ml), fetal calf serum (10%), sodium pyruvate (0.027mg/ml) and Gentamycin (0.05 mg/ml), pH 7.2-7.4 after equilibrating the solution at 5% CO_2 for 10 minutes, filter through 0.22 µ millipore filter. Five different combination of maturation media were

prepared following supplementation with hormones.

Group I : Medium supplemented with ovine LH (100 IU/ml).
Group II : Medium supplemented with Estradiol–17 beta (1 µg/ml).
Group III : Medium supplemented with Equine FSH (1 µg/ml).
Group IV : Medium supplemented with with ovine LH (100 Iµ/ml), Estradiol-17 beta (1µg/ml) and Equine FSH (1µg/ml)
GroupV : Served as control with no hormone.

The oocytes were picked up from washing medium and gently washed 3-4 times in 50 µ drops of corresponding maturation medium in sterilized petriplates. These oocytes were then transferred to respective maturation drop at the rate of 4-8 oocytes per drop and drops were covered with a thin layer of liquid parafin of spectroscopy grade. The petriplates were then placed in CO_2 incubator in an environment of 5% CO_2 in air with 95% humidity at 39^0c temperature for 24 hr. The oocytes were examined for enlargement of perivitelline space, expansion of cumulus mass and formation of polar body. The oocytes showing such changes were consider matured.

The asses the state of nuclear maturation on the basis of configuration of chromatin material ten oocytes from each group were processed by whole oocyte mounting method using glass slide and coverslip. Oocytes were fixed by dipping the slides into a coupling jar filled with acid-alcohol fixative (methanol : acetic acid, 3 : 1 v/v) for 24 hr. Thereafter Aceto-Orecin stain was dropped at one of the free end of coverslip through the space between slide and coverslip. The oocytes were stained for 5 minutes and the wet slides were examined under microscope for nuclear configuration.

RESULTS AND DISCUSSION

The in vitro maturation rate of buffalo oocytes were 57.81%, 68.75%, 77.22%,76.04% and 61.21% in Group I, II, III, IV and V, respectively. The results indicated that addition of LH in maturation medium lowered the maturation rate of buffalo oocytes nonsignificantly (57.81% vs 61.21%). The findings of Sirad et.al., (1992) support this observation. However, when estradiol is supplemented to the maturation medium the oocyte maturation rate was imporved nonsignificantly (68.75% vs 61.21%) which agrees with the findings of Totey et.al., (1993) and Bhatt (1995), Addition of FSH in maturation medium resulted in significant increase in oocyte maturation rate (77.22% vs 61.21%), the finding is in agreement with the observations made by Totey et.al., (1993) and Bhatt (1995), but when FSH was supplemented along with LH and estradiol the oocyte maturation rate was decreased nonsignificantly than that of FSH alone (77.22% vs 76.04%) and increased significantly as compared to group V. Most of other workers have also reported a significant increase in oocyte maturation rate when a combination of FSH, LH and estradiol were used for in vitro maturation of cattle oocytes (Younis et.al., 1989, Totey et.al., 1993, Sun et.al., 1994).

. Follicle stimulating hormone acts on the granulosa cells of small growing follicles to stimulate aromatisation of androgens to estrogens and it acts synergistically with estradiol to initiate LH receptor development on granulosa cells (Cran and Moor, 1980), FSH also enhances the maturation rate by suppressing the intra-cellular coupling of cumulus cells process to the oocytes (Moor et.al., 1981) and thereby inhibiting the meiosis inhibitory products to transmit from granulosa cells into the oocytes (Thibault et.al., 1987).

Oocytes from each group exhibiting maturation changes following in vitro culture. The nuclear configuration in these oocytes was in metaphase-II stage, after 24 hr of in vitro maturation. This was in agreement with the studies of Singh and Majumder (1992), Bhatt (1995) and Nag (1995) in buffalo.

In the light of the present investigation it can be concluded that supplementation of FSH alone in maturation medium results in highest rate for in vitro maturation of buffalo immature oocytes.

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EFFECT OF LOW DOSES OF PROSTAGLANDIN $F_2\alpha$ THROUGH INTRAVULVO SUBMUCOSAL (IVSM) ROUTE ON OESTRUS INDUCTION AND FERTILITY IN CROSSBRED COWS

K. KRISHNAKUMAR [1] and A. SUBRAMANIAN [2]

Department of Obstetrics and Gynaecology
Madras Veterinary College
Chennai – 600 007.

ABSTRACT

Twenty crossbred cows were randomly divided into two equal groups (I and II) and treated with 10 and 5 mg of $PGF_2\alpha$, respectively through intravulvo submucosal route between 10 and 12th day of the oestrus cycle. From this study, it is concluded that 5 mg of $PGF_2\alpha$ by IVSM route was as effective as that of 10 mg of $PGF_2\alpha$ in terms of oestrus induction and fertility rate in crossbred cows.

Many researchers reported that a low dose of $PGF_2\alpha$ by IVSM route can be used for estrus induction in bovines (Holy,1984; Neduncheralathan and Kathiresan, 1986). The present study was formulated to assess the effect of IVSM injection of 10 and 5 mg of $PGF_2\alpha$ on oestrus induction and fertility in cross bred cows

MATERIALS AND METHODS

Twenty crossbred cows after 60 days post partum were selected and randomly divided into two equal groups (I and II groups). I and II groups were treated with 10 and 5 mg of $PGF_2\alpha$ through IVSM route ipsilateral to the ovary bearing corpus luteum between day 10 and 12 of the oestrus cycle. The treated cows were observed for oestrus signs at 6.00 am and 6.00 pm daily for 30 minutes. Rectal palpation was done 24 hours after $PGF_2\alpha$ injection and at every 12 hours interval to confirm estrum. The post treatment oestrus percentage, onset, duration and intensity of oestrum were studied. The intensity of estrum was scored as per the method of Rao and Rao. (1981) with slight modification. Artificial insemination was done at 72 and 96 hours of post treatment. Conception was confirmed by rectal palpation in all the treated cows 45 days post insemination and the first service conception rate was calculated. The data was statistically analysed (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

The post treatment oestrus induction in the present study was 100 and 80 per cent with I and II groups,

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Part of the thesis approved for MVSC degree by the Tamilnadu Veterinary and Animal Science University, Chennai-600 007.

Present Address :
1. Assistant Professor, Department of Obstetrics and Gynaecology, Veterinary College and Research Institute , Namakkal-637 001.
2. Professor, Department of Biotechnology Madras Veterinary College, Chennai. 7.

respectively. The values were not found to be significant. The results observed in the present study concurred with the observation of Tarig Ahmed et.al., (1985). But the post treatment oestrus response in this study was higher than the observation of Narasimha Rao and Venkatramiah (1990). The overall time taken for the onset of estrum was 54.40 ± 1.33 and 56.63 ± 1.63 hours for the I and II groups, respectively. There was no significant difference in onset of oestrus between the I and II groups. The quick onset of oestrum in the intravulvo submucosal route compared to intramuscular route was attributed to the less distance between the site of deposition and action of PGF_2 α (Chatterjee et al., 1989). The mean duration of induced oestrum was 20.8 ± 0.40 and 20.5 ± 0.40 hours in respective groups. But there was no significant difference (P<0.05) between the groups which was in accordance with the findings of Pawshe et.al., (1991). The cows exhibited intense, intermediate and weak estrus was 60,40, and 0 per cent in group I and 37.5, 37.5 and 25 per cent in group II, respectively and there was no significant difference between the groups in oestrus intensity. The results in the present investigation was comparable with the observation of Chatterjee et.al., (1989). The first service conception rate for the induced estrus was 60 and 50 per cent respectively for I and II groups. The values were significant statistically and were comparable with the observation of Chatterjee et.al., (1989) and higher than the Chauhan et.al., (1986).

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SUPEROVULATORY RESPONSE IN IMPORTED HOLSTEIN FRIESIAN COWS UNDER FIELD CONDITIONS

B.M. RAVINDRANATHA and S.M. REDDY

Department of Gynaecology and Obstetrics
Veterinary College, University of Agricultural Sciences,
Hebbal, Bangalore – 560 024.

ABSTRACT

Fourteen cycling purebred Holstein Friesian cows maintained by farmers in villages were superovulated using Folltropin-V administered intramuscularly in eight divided doses over a period of four days. The embryos were collected non-surgically from donors after 72 hrs. post breeding. The Superovulation response was recorded in terms of mean number of anovulatory folicles (3.78 ± 0.35), corpora lutea (8.57 ± 0.91) and mean total response (12.35 ± 1.14). Mean number of embryos recovered, freezable, non-freezable and unfertilized ova were 5.14 ± 0.86, 2.21 ± 0.46, 2.92 ± 0.68 and 1.07 ± 0.26 respectively.

Superovulation in cattle has been successfully attempted since several decades with great variation in response. The variability has been attributed to a variety of factors such as, age of the cow (Hasler et.al., 1983), breed (Lindshell et.al., 1985), environmental conditions and plane of nutrition (Datta et.al., 1992),the type of hormone used (Monneaux et.al., 1983) and the endocrine status of the animal (Kharche et.al., 1997). Since limited information is available on the superovulatory response of imported Holstein Friesian cows under field conditions, the present study was aimed to study the superovulatory responses using Folltropin-V.

MATERIALS AND METHODS

Fourteen imported Holstein Friesian cows of high performance between first and third lactation were selected. All the cows had normal calvings 60-120 days before superovulation without any history of dystocia or post parturient complications. The superovulation was induced on day-10 of the second synchronized oestrus by intramuscular administration of 400mg of NIH FSH (Folltropin-V, Vetrepharm Inc.Canada) in eight divided doses at 12hrs interval in descending doses (60/60,55/55,45/45 and 40/40). Twenty Five milligrams of lutalyse was administered i/m along with the sixth dose of FSH (day 12). The superovulatory response was assessed on day six of superovulatory oestrum by rectal examination of ovaries to ascertain the number of anovulatory follicles, number of corpora lutea on both the ovaries. The embryos were flushed on day 6.5 – 7.0 of superovulatory oestrum using modified phosphate Buffered saline. Number of embryos recovered, their quality i.e (freezable and non

freezable) were assessed as per methods described by Reddy (1994).

RESULTS AND DISCUSSION

All the 14 donors exhibited superovulatory response to Folltropin-V. The number of anovulatory follicles and corpora lutea, were 3.78 ± 0.35, 8.57 ± 0.91, respectively. An average of 5.14 ± 0.86 embryos were recovered per donor of which 1.21 ± 0.28 were excellent and 1.00 ± 0.18 good which were considered as freezable quality. The non-freezable embryos per donor respectively. The mean number of unfertilized ova accounted for 1.07 ± 0.26 per donor.

In the present study mean number of corpora lutea in response to superovulation (8.57 ± 0.91) was higher than those reported by Becker and Pinherio (1986) and Dutta et.al., (1992) who reported 7.10 ± 0.90 and 4.20 ± 0.80 respectively.

The mean anovulatory follicles recorded (3.78 ± 0.35) in the present study in response to Folltropin-V are in conformity with the findings of Laurincik (1993) who reported 3.3 ± 0.5 anovulatory follicles. The higher mean corpora lutea recorded in the present study might have been the result of meticulous control of donor cycles through double dose of PGF_2 α and probably the batch of hormones used. Similar reasons were also attributed to the variation in superovulatory response such as age (Hasler et.al.,1983), type of hormones employed for superovulatory treatment (Monniaux et.al.,1983) breed (Lindshell et.al., 1985) season (Hasler et.al., 1983) and day of initiation of superovulatory treatment (Moor et.al., 1984).

The total response to superovulation in donors including anovulatory follicles and corpora lutea in the present study was 12.35 ± 1.14 per donor, which closely correlates with observations made by Pawshe et.al., (1992) in dairy cows.

The mean number of embryos recovered in the present study (5.14 ± 0.86) are in conformity with those reported by Gabrikov and Dronin (1991) who recorded 5.3 . ± 0.63 embryos. Mean number of freezable embryos recovered in the present study (2.21 ± 0.46) are similar to those reported by Walsh et.al., (1993) who recorded 2.1 ± 0.7.

Since the donors were selected from among cows maintained by dairy farmers in rural areas, inspite of the guidance provided to them on management of donors, the type of maintenance and nutritional status varied greatly between individual cows of farmers, which might have contributed for variation in quality of embryos recovered.

The mean number of unfertilized ova recorded in the present study (1.07 ± 0.26) is lower than those reported by Laurincik et.al., (1993), who recorded 2.3 ± 0.3. It is concluded that the superovulatory response in imported Holstein Friesian cows under field conditions is comparatively lower than those reported by authors in the western world.

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EFFECT OF eCG ON FERTILITY IN NORGESTOMET PRIMED POSTPARTUM ANOESTRUS COWS*

S.SELVARAJU[1] and R.C.RAJASUNDARAM[2]

Department of Obstetrics and Gynaecology
Madras Veterinary College, Chennai.

ABSTRACT

Sixteen healthy postpartum anoestrus crossbred cows were divided into two groups as NOR and NOR + eCG. In both the groups oestrus was induced with 3 mg. Norgestomet ear implant combined with crestar injection containing 3 mg norgestomet and 5 mg oestradiol valerate (Day 0). In NOR group drug was administered at the time of implant removal (Day 9) whereas in NOR + eCG 500 IU eCG was administered intramuscularly. AI was carried out 8 to 12 hours after the onset of oestrus with good quality frozen semen. The first service (25 Vs 12.5 per cent) and overall (62.5 Vs 25 per cent) conception rate were higher in NOR + eCG group. It was concluded that administration of eCG at the time of implant removal was found to be superior than norgestomet alone to improve fertility response in postpartum anoestrus cows.

Ovarian activity can be induced successfully with progestogen treatment (Peters and Lamming, 1986), but the conception rate after induced oestrus is reported to vary. To improve conception rate at the induced oestrus, gonadotrophin has been frequently used with progestogen to stimulate follicular growth and ovulation with good oestrus response (Mulvehill and Sreenan, 1977 ; Umed Singh, 1995). Hence the present study was formulated to assess the effect of eCG on fertility in norgestomet primed postpartum anoestrus cows.

MATERIALS AND METHODS

Jersey crossbred parous cows maintained under standard feeding and management condition at Livestock Research Station, Kattupakkam, TANUVAS, were utilised for this study. Sixteen apparently healthy postpartum cows above three years of age, weighing between 300 to 400 kg with the history of normal calving, not resumed oestrus within 60 days postpartum and free from any palpable reproductive tract abnormalities were selected for this study. Rectal examination was done twice at 10 days interval and the animals not having any palpable structures in both the ovaries were confirmed as postpartum anoestrus. Selected animals were randomly divided into two equal groups namely, NOR and NOR + eCG. Both the groups were treated with 3 mg norgestomet ear implant (Crestar, Intervet, Holland). At the time of implant insertion (Day 0), all the cows were administered 2 ml crestar, injection containing 3 mg norgestomet

* Part of M.V.Sc. Thesis of first author submitted to TANUVAS, Chennai.

1. Ph.D.Scholar IVRI, Izatnagar.
2. Associate Professor, Department of clinics, MVC Chennai.

and 5 mg oestradiol valerate intramuscularly. The implant was removed on day '9' of the experiment. The NOR group (n=8) was taken as control. In this group, norgestomet ear implant was administered with intramuscular crestar injection and on day '9' the implant was removed. In NOR + eCG group (n=8), 500 IU eCG (Folligon, Intervet, Holland), was administered intramuscularly at the time of implant removal.

Oestrus detection was done at 6 AM, 12Noon, 6 PM and 10 PM daily after implant removal. The experimental cows were bred with AI using good quality frozen semen at 8 to 12 hours after the onset of oestrus. The cows that did not conceive at the induced oestrus were observed subsequent two cycles and inseminated. Overall conception rate was calculated by percentage of females conceived of the total inseminated. Pregnancy was confirmed by palpation per rectum at 60 days post insemination.

RESULTS AND DISCUSSION

All the animals treated in NOR + eCG group came to oestrus and were inseminated. In NOR group, first service conception rate (12.5 per cent) obtained was in agreement with Hixon et.al. (1981) and Beal et.al. (1984). In the postpartum anoestrus cows, presence of luteal phase plasma progesterone, prior to oestrus for a period as short as 7 day will allow normal fertility, presumably because it provides adequate priming (Munro, 1989). The dosage of progestin in implant appears to be subluteal (Kesler and Favero, 1995) it may not provide adequate priming and may depress fertility in postpartum aneoestrus cows. Improvement in conception rate to first service (25 percent) after administration of eCG is in accordance with the reports of Mulvehill and Sreenan (1977) and Colombani et.al. (1979). However Smith et.al. (1979) reported no improvement after administration of eCG. Improvement in conception rate after administration of eCG may be due to coincidence of FSH and LH peak (Narasimha Rao and Suryaprakasam, 1991).

The overall conception rate of 25 per cent obtained in NOR group is in close agreement with Hixon et.al. (1981). However, Kiser et.al. (1980) and Balasubramanian (1989) reported more than 50 per cent overall conception rate in the postpartum cows. Two animals that reverted back to anoestrus may contribute to the lower conception rate in the present study. NOR + eCG group had higher overall conception rate of 62.5 per cent which is in agreement with Umed Singh (1995). However, Luthra et.al. (1994) reported more than 80 per cent conception rate. This may be due to the effect of eCG in regulation and synchronization of endocrine event and promotion of follicular maturity in subsequent cycles (Narsimha Rao, 1991)

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TRANSFERRIN POLYMORPHISM AND THEIR CORRELATION WITH TRAITS OF ECONOMIC IMPORTANCE IN MURRAH AND BERARI BUFFALOES *

V.R.MAJ BALLEWAR ** S.Z. ALI *** and A.R.SIROTHIA ***

Department of Animal Management, Hygiene,
Genetics and Breeding and Extension Post Graduate Institute,
Punjabrao Krishi Vidyapeeth, Akola, (M.S.)

ABSTRACT

In the present study six transferrin types were found in the decreasing order of their mobility i.e. TfDD, TfDN, TfDN, TfNN, TfNK and TfKK which were controlled by three codominant alleles i.e TfD, TfN and TfN. Age at first calving was non-significantly correlated in both the breeds. Lactation length both in Murrah and Berari buffaloes were positively correlated with distance of migration. The lactation yield was significantly correlated in Berari buffaloes. Dry period in Murrah buffaloes was positively significantly correlated with distance of migration of transferrin but not in Berari buffaloes. Intercalving period in both the breeds was found to be statistically not correlated. It was therefore suggested that the selection for faster migration transferrin types i.e TfDD, TfDN and TfDN with due consideration to standard selection procedures may improve the lactation in Berari buffalo.

Polymorphism in farm animals have been found to be associated with milk yield (transferrin types) butter fat percentage (blood groups), host parasite relationship, advantage of one gene over the other in particular environment (haemoglobin and potassium) and fertility (transferrin types) (Reddy.1969).

The most pronounced genetic variability has been reported in the B-globulin fraction of the serum proteins. Variation in B-globulins are due to the iron carrying protein substance called transferrin (Gilbert 1959). The B-globulin phenotype of an individual remain constant and has no effect of age (Ashton et.al., 1967). The between breed variation was found to be in agreement with Hardy Weinberg equilibrium hypothesis (Carr, et.al., 1966 : Khanna and Singh 1978). Various studies have shown that B-globulin locus is concerned in the genetic control of milk yield (Larson et.al., 1956; Ashton, 1960; Jamieson et.al., 1967). Kiddy et.al., (1975) studied the transmitting ability of TF type in bulls and suggested that it may be of little value in selection of bulls but it may be useful eventually if other markers related to producing ability can be identified and used in conjunction with it and pedigree information.

The present study was undertaken with the objective to identify the correlationship between

* Part of MVSc thesis submitted to Punjabrao Krishi Vidyapeeth Akola, Maharashtra by first author.

** Instructor, Animal Genetics and Breeding, RVC centre and School. Meerut Cantt. (UP).250 001

*** Associate Professors, Deptt, of Animal Genetics and Breeding. F. H. V. Akola. Maharashtra. 444 104.

various transferrin types and the traits of economic importance in buffaloes.

MATERIALS AND METHODS

The experiment was conducted at Livestock Instructional Farm, and the University Department of Animal Genetics and Breeding, PKV Akola. The Murrah and Berari buffaloes were taken as experimental material. The transferrin typing was carried out by standard method as described (Ballewar et.al., 1997). The data pertaining to economic traits such as age at first calving, lactational length, lactation milk yield, dry period and intercalving period used for this study were collected from the pedigree and breeding record pertaining to the experimental animals, maintained at this farm.

RESULTS AND DISCUSSION

In the present study, six transferrin types were found in the decreasing order of their mobility and were classified as TfDD, TfDN, TfDK, TfNN, TfNK, and TfKK, controlled by three codominant alleles TfD, TfN and TfN. (Ballewar et.al.,1997). The results are summerised in the table.

Table Correlation coefficients between distance of migration and traits of Economic importance in Murrah and Berari buffaloes.

Breed	Age al first calving	Lactation length	Lactation yield	Dry period	Intercalving period
		**	NS	**	NS
Murrah	0.2218	0.9094	0.2785	0.8929	0.5496
	(26)	(22)	(22)	(20)	(7)
	(1522 Γ 40)	(308 Γ 15)	(644 Γ 42)	(277 Γ 4)	(534 Γ 56)
		**	**	NS	NS
Berari	***	0.9612	0.9555	0.0952	0.1171
		(27)	(27)	(26)	(16)
		(318 Γ 9)	(838 Γ 34)	(300 Γ 50)	(570 Γ 37)

Note: ** ➢ Significant at 1 % level of significance.
*** ➢ Data not available on age at first calving.
NS ➢ Not significant.
Values in parenthesis indicate the no. of pairs.

It was observed that the 'r' between distance of migration and age at first calving in Murrah buffaloes was found to be statistically non significant, indicating that age at first calving and distance of migration of transferrin are not associated.The lactation length in both Murrah and Berari buffaloes was postitively correlated with distance of migration indicating that an increased distance of migration significantly increase the lactation length of the animal.

The lactational yield in Murrah was not statistically associated with

distance of migration of transferrin whereas in Berari buffaloes it was significantly positively correlated indicating that selection for faster migrating TF types i.e DD, DK, DN in Berari buffaloes may improve the lactational yield significantly.

The correlation coefficient between distance of migration of transferrin and dry period indicated that in Murrah buffaloes dry period is positively and significantly correlated where as in Berari buffaloes it was not associated with distance of migration of transferrin. The intercalving period in both Murrah and Berari buffaloes was found to be not statistically correlated with distance of migration of transferrin.

The present findings of association of faster migrating transferrin with milk yield in Berari buffaloes are in agreement with the findings of Zwiaver (1981). Chaudoba et al., (1981) and Skvortesov et. al., (1981) as they reported that type TFDD was found to be superior in average milk yield, peak lactation in cattle.

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OVARIAN RESPONSE AND STEROID PROFILE IN CROSSBRED SHEEP TREATED WITH PREGNANT MARE SERUM GONADOTROPHIN

SURESH S. HONNAPPAGOL [1], PRAKASH NADOOR [2*], and DEEPAK DESHPANDE [3]

Veterinary College, University of Agricultural Sciences, Dharawad
Post Box No.6, Bidar, Karnataka 585 401.

ABSTRACT

Ovarian response and steroid profile were studied in 16 crossbred ewes. Eight ewes were treated with PMSG on Day 10 and remaining were kept as control. At laprotomy, the PMSG treated ewes showed 2.2 ± 0.95 corpora lutea and more number of matured follicles as against 0.7 ± 0.15 corpora lutea and no matured follicles in control ewes. PMSG treated ewes had a significantly ($P \leq 0.05$) higher estradiol (65.10 ± 5.6 pg/ml) on the day of superovulatory estrus than control ewes (18.00 ± 6.15 pg/ml). The paper discusses the probable role of PMSG in follicular selection and estrogen synthesis by follicles thereby increasing ovulation rate.

Faster propagation of offspring involves induction of "superovulation" using exogenous gonadotrophic hormonal preparation like Pregnant mare serum gonadotrophin (PMSG), Follicle stimulating hormone (FSH) and Human chorionic gonadotrophin (hCG) in ewes. However, the ovarian response to these superovulatory drugs varies with initiation of treatment and dose (Ryan, 1984) and is unpredictable on most occasions due to several exogenous and endogenous factors associated with it, more particularly in sheep, due to difference in follicular dynamics and ovulation rate among different breeds. (Cahill et.al., 1981, Draincourt et.al., 1985). Reports on use of superovulatory drugs in triple crossed sheep (Bannur x Deccani x Southdown) is not documented. Hence the present study was designed to assess the ovarian response, ovulation rate and steroid profile in crossbred sheep treated with PMSG during mid luteal phase of induced estrous cycle.

1. Prof essor and Head, Department of Animal Reproduction
2. Asst. Prof., Department of Pharmacology
3. Asst. Prof.essor Department of Physiology.
* Author for correspondence

MATERIALS AND METHODS

Experiment was initiated during late winter season. The adult ewes were selected (n=16) from the sheep flock maintained at Veterinary College. Bidar under normal environment conditions. The animals were synchronized for estrus with two doses of $PGF_{2\alpha}$ analog, cloprostinol (Estrumate, U.K.) given at a dose rate of 100µg per animal intramuscularly, 11 days apart. The onset of estrus was confirmed by introduction of a ram in the flock. The day of estrus was designated as Day 0 and on Day 10 of the estruous cycle, PMSG (Folligon, Intervet, Holland) was administered to eight ewes (treatment group) at a dose

of 600 IU intramuscularly and the remaining ewes (n=8) received phosphate buffer saline (control). Forty-eight hours later all the animals were treated with $PGF_{2\alpha}$ (100µg IM) and later observed for the onset of estrus as described above.

The animals were allowed for natural breeding and six days later laprotomy was performed by mid-ventral approach (Acepromazine maleate 0.1 mg/kg. IM ± 2% Lidocaine as local anaesthetic) to asses ovulation rate. Two animals randomly drawn from each group underwent laprotomy between 24-30 h after the onset of estrus to assess follicular activity.

Blood samples were drawn during synchronization and superovulatory protocols to be used for steroid hormone profile. Plasma progesterone (P_4) and estradiol (E_2) concentration were measured by radioimmunoassay (RIA) using standard and validated method (Prakash, 1989; Prakash et.al., 1995). Student 't' test was employed to compare the data and all the values were expressed as Mean ± SE.

RESULTS AND DISCUSSION

At the onset of synchronized estrus, the plasma P_4 concentration was 0.43 ± 0.12 and 0.47 ± 0.27 ng/ml, which was increased to 2.13 ± 0.35 and 2.01 ± 0.42 ng/ml on Day '10' in control and PMSG treated group respectively.

The superovulatory estrus was also marked by reduced plasma P_4 (0.36 ± 0.08 Vs 0.41 ± 0.09) concentration in both control and treated goups. The P_4 concentration in both the groups did not differ significantly on any day. Plasma E_2 concentration was 19.10 ± 0.95 and 22.26 ± 1.20 pg/ml on Day '0' of the estrous cycle in the control and PMSG treated groups and there was no significant difference ($P \geq 0.05$). On the day of superovulatory estrus, the plasma E_2 concentration was significantly ($P \leq 0.05$) higher in PMSG treated ewes (65.10 ± 5.60 pg/ml) compared to control ewes (18.0 ± 6.15 pg/ml). Within the groups E_2 concentration was significantly ($P \leq 0.05$) higher on superovulatory estrus compared to Day '0' of normal cycle in PMSG treated group while no significant difference was noticed in control ewes.

The ovulation rate as assessed by number of corpora lutea revealed that animals treated with PMSG had 2.20 ± 0.95 corpora lutea compared to 0.7 ± 0.15 in control ewes and this was found to be significant ($P \leq 0.05$). PMSG treated ewes had signifiantly ($P \leq 0.05$) more number of matured follicles (1.5 ± 0.34 per animal) contrary to the control ewes which did not have any matured follicles.

Treatement with PMSG at a dose of 600 IU on Day '10' of the estrous cycle increased the ovarian activity as confirmed by high E_2 concentration and multiple corpora lutea in the treatment ewes. Pharmacologically PMSG has a very long half-life compared to pituitary gonadotrophins and thereby resulting in abnormal endocrine status (Monia

et.al., 1983).The presence of unovulated follicles in PMSG treated ewes in the present study may be attributed to PMSG interfering with the normal ovulatory LH surge as reported earlier. Higher ovulatory response indicates the probable occurrence of a period of emergence of follicles during that period. Our treatment regimen coincides with period II of follicular emergence (Deshpande, 1997) which lies between Day 8 or 9 and Day 13 or 14 of the estrous ecyle in Bannur ewes. Hence, PMSG might have influence on recruitment or selection process during folliculogenesis (Dott et.al., 1979 McNatty et.al., 1982). Increased E_4 concentration induced by PMSG hints at its stimulatory effect on E_2 synthesizing ability of the follicles. Exogenous gonadotrophin supplementation during mid luteal phase enhances ovulation rate and the strength of the stimulus determines the number of follicles responding and their inherent ability to repond (Ireland, 1987) and there appears to be variation in ovulatory response with respect to dose of PMSG. However, high dose of PMSG may found to exert detrimental effect on maturation of follicles (Draincourt, 1987).

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QUALITY OF OOCYTES OBTAINED FROM BUFFALO OVARIES DURING WINTER AND SUMMER MONTHS

S.K. SINGLA, R.S. MANIK, M.S. CHAUHAN and M.L. MADAN

Embryo Biotechnology Centre
National Dairy Research Institute, Karnal 132 001.

ABSTRACT

A total of 2296 buffalo ovaries were collected from slaughter house and subjected to the study. The percentage of three grades (Grade I, II, and III) of oocytes classified on the basis of cumulus cell layers and morphology during winter were significantly ($p< 0.01$) different than during summer. Pooled utilizable oocytes of grade I and II during winter and summer were 62.4 and 77.3 per cent respectively.

Estrus cyclicity, recruitment of follicles and reproductive efficiency in buffaloes is greatly affected by the month of the year or the season (Madan and Raina, 1984; Madan et.al., 1988; Madan, 1990, 1992; Taneja et.al., 1995). Also there are no reports on quality of oocytes obtained from abattoir ovaries of buffaloes during different seasons. Thus a study was planned for the assessment of quality of oocytes obtained from post-mortem buffalo ovaries during two distinct seasons. i.e. summer and winter.

MATERIALS AND METHODS

Individual buffalo ovaries were aspirated for oocytes using a needle of 19 gauge and a plastic syringe in modified Dulbecco's Phosphate Buffered Saline with 0.4 per cent lyophilized bovine serum albumin. The needle was withdrawn as far as possible and the tip shifted from one follicle to another keeping the negative pressure intact. All visible follicles were aspirated and shifted to 100x100 mm searching dish with gird. The oocytes were searched, washed and separated into three grades i.e good (grade-I), fair (grade-II) and poor (grade-III) on the basis of cumulus cell layers and morphology. The total oocytes obtained and their gradewise distribution was recorded.

RESULTS AND DISCUSSION

As seen in Table, a total of 3,210 oocytes were obtained from 2,296 ovaries. They were isolated and thoroughly examined for their gradation as grade I, II or III. Total ovaries handled during each trial ranged from 15 to 171 and respective figures for oocytes handled per trial ranged from 27 to 239.

A total of 834, 1486 and 890 oocytes of grade I, II and III respectively were obtained from 2,296 ovaries. The number of grade-I oocytes ranged from 7 to 73. The number of grade-II and grade-III ranged from 2 to 126 and 5 to 71 respectively. This data included both ovaries with corpora lutea and without corpora lutea.

Table:1 Number and grade of oocytes obtained through aspiration from ovaries during winter and summer months.

Season	No of trials	Ovaries	Oocytes Grade I (%)	Grade II (%)	Grade III (%)	Total (%)
Winter	12	Total 594	331 (35.4)[a]	252 (27.0)[c]	352 (37.6)[c]	935 (100.0)
		Range15-94	12-55(26.2-50.0)	2-38(7.4-40.4)	13-46(31.4-53.2)	27-118
Summer	17	Total 1702	503 (22.1)[b]	1234 (54.2)[d]	538 (23.7)[f]	2275 (100.0)
		Range 30-171	7-73(8.0.37.0)	19-113(37.0-69.5)	5-71(13.8-37.5)	32-239
Pooled	29	Total 2296	834 (26.0)	1486 (46.3)	890 (27.7)	3210 (100.0)
		Range 15-171	7-73(8.0-50.0)	2-126(7.4-67.5)	5-71 (13.3.53.2)	27-239

Different superscripts in the same column differ significantly ($p<0.01$)

Critical appraisal of Table suggests that grade-I oocytes were 35.4 per cent and 22.1 per cent respectively for winter and summer months. The respective range of per cent grade – I oocytes during different trials were 26.2 to 50.0 and 8.0 to 37.0 per cent for the two seasons. The statistical analysis of data of per cent grade – I oocytes obtained during winter and summer suggests that they were significantly different during the two seasons at one per cent level of significance. Further grade-II oocytes were more during summer months than during winter and the respective average for summer and winter were 54.2 and 27.0 per cent. The values were highly, significant at one per cent level of significance. The grade-III oocytes during winter and summer were 37.6 and 23.7 per cent respectively with a respective ranges of 31.4 to 53.2 and 13.8 to 37.5 per cent. The per cent grade – III oocytes between the two seasons was again significantly different even at one per cent level of significance. Pooled utilizable oocytes of grade 1 and II during winter and summer were 62.4 and 77.3 per cent respectively.

The number and grade of aspirated oocytes obtained from buffalo ovaries during two seasons were significantly different ($p<0.01$)for all the three grades of oocytes (Table). Similar season-wise data is not available in buffaloes, though some reports presenting data on different grades during one single season (Suzuki et.al., 1992; Jain et.al., 1995; Das et.al., 1996) are available. Our results show that 26 per cent of total oocytes aspirated were of grade I, whereas Suzuki et.al., (1992). Jain et.al., (1995) and Das et.al., (1996) have reported 9.5. 11.1 and 1.25 per cent of grade - I oocytes, respectively. The same authors have reported grade II oocytes to the tune of 17.0. 37.8 and

43.7 per cent, respectively, whereas in our data (pooled for two seasons), 46.3 per cent grade II oocytes were obtained. Similarly grade III oocytes as reported by Suzuki et.al., (1992), Jain et.al., (1995) and Das et.al., (1996) were 55.2, 51.1 and 50.0 per cent, respectively as compared to our compilation of 27.7 per cent. Though the results presented by three workers are not strictly identical to those reported in this study but then the method of grading the oocyte was also not similar in terms of the grade of oocytes. The variability in results from trial to trial can be ascertained from the wide range of percentage of grade I, II and III oocytes as 8.0 to 50.0. 7.4 to 69.5 and 13.8 to 53.2 respectively.

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MORPHOLOGICAL AND MORPHOMETRICAL STUDIES ON FOLLICULAR OOCYTES OF BUFFALOES

B.C. SARKHEL [1]. R. VINZE [2] and H.K.B. PAREKH [3]

College of Veterinary Scinece & Animal Husbandry
Jawharlal Nehru Krishi Vishwa Vidyalaya. Jabalpur,
Madhya Pradesh – 482 001.

ABSTRACT

A study was undertaken on 47 pairs of ovaries collected from buffalo slaughter house. There was not much variation in size measurements and weight of right and left ovaries. The average number of observed follicle was 9.07 per ovary, which did not differ much in right and left ovaries. The number of oocytes recovered from right ovary was slightly higher (2.53) than left (2.38). The incidence of good quality oocytes (Grade I and II) recovered was less (39%) than poor quality (Grade III and IV) oocytes (61%). The overall mean diameter of oocytes was 226.75 µm with cumulus and 148.69 µm without cumulus. There was a highly significant difference in diameter of different grades of oocytes with and without cumulus. Similarly there was a highly significant difference in follicle area for different grades of oocytes.

In India due to disorganized buffalo breeding large number of good quality buffaloes are being slaughtered every year. The ovaries of these slaughtered animals can be a very valuable and inexpensive source for embryo production (Pineda and Bowen 1980) which can be used for embryo transfer in buffaloes even during nonbreeding season.

Leibfried and First (1979) reported a direct relationship between morphological characteristics of follicular oocytes and their ability to mature in vitro. In view of the above the present research work was undertaken to study the rate of recovery and morphometrical characteristics of different grades of oocytes recovered from different sizes of follicles.

MATERIALS AND METHODS

The ovaries from 47 buffaloes were collected from slaughter house and brought to the laboratory in phosphate buffered saline (PBS). The following measurements were taken within 2 to 3 hour of slaughter.

1. Weight of right and left ovaries.
2. Length, width and thickness of ovaries (by dial calipers)
3. The follicular areas were measured by a dial calipers having 0.05 mm accuracy. As the shape of the follicles were round or oval, as per standard mathematical technique the area of an oval or round structure is calculated by the following formula.

Present address :
1. Associate professor. Dept. of Animal Breeding And Genetics.
2. M.V.Sc. & A.H.sudent.
3. Professor & Head .Dept of Animal Breeding & Genetics.

$$\text{Approx.follicular area} = \Pi \frac{\text{Max.Length X Max.width}}{4}$$

The oocyte from each measured follicle was aspirated by 5 ml disposable syringe and 21 gauge needle. The oocytes in PBS were poured into a Falcon petri dish and examined under stereozoom microscope. The oocytes were classified into four grades (I to IV) based on presence or absence and number of layers of cells around zona pelucida (Singh, 1992)

The follicles were divided into four groups as per their size. FA4 : 3 to 6 sq. mm area; FA3 : 6 to 12 sq. mm area; FA2 : 12 to 18 sq. mm area and FA1 : above 18 sq. mm area.

The different grades of oocytes recovered by the puncture of different groups of follicles were recorded. The oocytes were measured by ocular micrometer standardized with stage micrometer. The data were analysed statistically.

RESULTS AND DISCUSSION

The weight, length, width and thickness of right and left ovaries were 3.31± 0.23, 2.05 ± 0.61, 1.53 ± 0.49, 1.17 ± 0.40 and 3.25 ± 0.25, 2.05 ± 0.56, 1.49 ± 0.50, 1.14 ± 0.50 respectively. The number of follicles per ovary varied from 3 to 15 with an average of 9.30 though the average number observed in right ovaries were slightly higher (9.10) than the left (9.05).

The overall average number of oocytes recovered per ovary was 2.46. The number of oocyte recovered from right ovary was slightly higher (2.53) than the left (2.38). The incidence of grade III oocytes recovered was maximum (35.8%) followed by grade IV (24.7%) (Table.1). The incidence of Grade I and II oocytes were minimum, the values observed were 19.0% and 20.5% respectively. The grade I and II oocytes are considered as good quality oocytes.

Further, it was observed that 95 per cent of grade I and 85 per cent of grade II oocytes were recovered from medium size follicles whose area ranged from 6 to 12 sq.mm. (FA.2) and 12 to 18 sq.mm. (FA.3). On the other hand 84.2 per cent of grade IV oocytes were recovered from small follicles (FA.4). The very large follicles (FA.1) are also not reported to be suitable for follicle aspiration. Selvaraj et.al., (1992) also recovered lower percentage of good quality oocytes from buffalo ovaries.

The overall mean diameter of oocytes with and without cumulus was 226.75 ± 3.91 and 148.69 ± 0.9 respectively. (Table.2) There was a highly significant difference ($p<0.01$) in mean diameter of different grades of oocytes with and without cumulus, The LSD test indicated that the mean diameter of grade III oocytes with cumulus was significantly lower ($P<0.01$) than grade II and grade I oocytes. For oocytes without cumulus, there was no significant difference in mean values of grade II and III oocytes. However, grade I oocytes was significantly larger than all other

Table: 1 Grade of oocytes recovered from ovaries of buffalo

Attribute	Right		Left		Total
	No.	%	No.	%	No.
Ovaries examined	47	-	47	-	94
Visible follicles	423	48.45	450	51.55	873
Follicle punctured	247		250		497
Oocytes recovered					
Grade I (G I)	16	6.93	29	12.55	45
Grade II (G II)	27	11.69	20	8.66	47
Grade III (G III)	42	18.18	40	17.32	82
Grade IV (G IV)	27	11.69	30	12.99	57
Total	112	48.48	119	51.51	231
Mean No.of oocytes recovered/ovary	2.38		2.53		2.46
Percentage oocyte recovered out of follicle puncture		45.34		47.60	46.48%
Different grades of Oocytes recovered from Different groups of follicles.					
	G.I		G.II	G.III	G.IV
FA. 4 (3 to 6 sq.mm)	1 (2.2%)		2 (4.3%)	29 (35.4%)	48 (84.2%)
FA. 3 (6 to 12 sq.mm)	16 (35.6%)		20 (42.5%)	30 (36.6%)	7 (12.3%)
FA. 2 (12-18 sq.mm)	25 (55.5%)		20 (42.5%)	17 (20.7%)	2 (3.5%)
FA. 1 (18 sq.mm and Above	3 (6.7%)		5 (10.6%)	6 (7.3%)	0 (0%)

Table.2 Mean and SE of different grades of oocytes with respective follicles in buffalo

Grade	Oocyte (um)		Follicle (sq.mm)
	With cumulus	without cumulus	area
I	287.24 [a] + 5.05	155.02 [a] + 1.97	27.02 [a] + 1.33
II	229.45 [b] + 4.94	149.70 [b] + 1.93	16.70 [b] + 1.30
III	192.00 [c] + 3.74	148.29 [b] + 1.46	9.40 [c] + 0.98
IV	-----	143.44 [c] + 1.75	5.85 [d] + 1.18
Overall mean	226.75 + 3.91	148.69 + 0.90	13.44 + 0.77

Means having same superscript did not differ significantly.

three groups. Likewise mean diameter of grade IV oocyte was significantly smaller than all other three groups (Table.2).

The overall mean follicular area observed in the present study was 13.44 + 0.77 sq. mm. There was a highly significant difference ($p<0.01$) in mean follicular area possessing different grades of oocytes. The area of grade III follicles (27.02 sq.mm) was significantly higher ($p<0.01$) than the rest. Likewise the area of grade IV follicles was significantly lower (5.85 sq.mm) than the rest. The mean diameter of grade I,II,III and IV follicles decreased gradually, each differing significantly from each other (Table.2).

The mean oocyte diameter reported by Selvaraj et.al., (1992) in buffalo was lower than the present study. The oocyte diameter of the present study are comparable with the oocyte diameter of goat as reported by Singh (1992) and also with the oocytes of pigs as reported by Stanvic et.al., (1992).

A comparison of three grades of oocytes with cumulus in the present study revealed a gradual increase in diameter, each group differing significantly from each other. However, this pattern was not so difinite in oocytes without cumulus Thus it looks obvious, that significant difference in oocytes with cumules was due to cumulus layer only and not due to actual oocyte. This indicate that the size of oocytes recovered from ovaries by aspiration did not changed much during in vitro maturation and there is only significant nuclear changes occur with maturation.

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PROGNOSTIC IMPORTANCE OF CERTAIN BLOOD INDICES IN RELATION TO SURVIVABILITY OF THE BUFFALOES AFTER OBSTETRICAL TREATMENT

S. PRABHAKAR, A.S. NANDA and S.P.S. GHUMAN

Department of Animal Reproduction,
Gynaecology & Obstetrics Punjab Agricultural University,
Ludhiana – 141 004. India.

ABSTRACT

Blood indices viz., packed cell volume, haemoglobin, glucose, proteins and immunoglobulins were estimated, in dystocia affected buffaloes prior, during and for two days after obstetrical treatment. Increase in PCV and Hb and decrease in proteins and immunoglobulins during post treatment period indicate poor prognosis.

Dystocia and obstetrical treatment produce immense stress on the dam and impair various body functions. Mortality rate in such cases is high, which could be minimised by better understanding of these derangements and their prompt corrections. Packed cell volume and haemoglobin are useful indices for evaluation of alterations in plasma volume and electrolyte balance (Boyd, 1981). Degree of immune depression of the animal due to stress of calving abnormalities can be easily determined by plasma immunoglobulins and protein turnover (Yablonskii and Prigara, 1984). So, the present study has been planned to make thorough assessment of these blood indices in dystocia affected buffaloes, which may determine the prognosis of the dam.

MATERIALS AND METHODS

Twenty-six buffaloes presented for treatment of dystocia and which required fetotomy and/or caesarean section for relieving dystocia were included in the present study. Dystocia was relieved by partial/complete fetotomy in ten buffaloes and caesarean operation was done in remaining sixteen buffaloes. Blood samples were collected through jugular venipuncture before the start of any obstetrical treatment, during treatment and once daily thereafter, on two post-treatment days. Three millilitre of blood was separated for haematological indices and for preparation of protein free filtrate for determining blood glucose. The procedures followed for the assay of blood indices were; packed cell volume and haemoglobin (Schalm et.al., 1975). Glucose (Frankel et.al., 1970) proteins and immunoglobulins (Lowry et.al., 1951). Statistical analysis was done by student's t-test (Gupta, 1986)

RESULTS AND DISCUSSION

Out of total twenty-six, twelve buffaloes survived on the post-

treatment days. Nine buffaloes died immediately after obstetrical treatment and remaining five buffaloes died by three to four days following manoeuvring. The buffaloes which died after obstetrical manipulations had higher Packed cell volume and haemoglobin concentrations on post treatment days than the surviving counterparts. Atwal (1993) also recorded higher PCV and Hb values in buffaloes which died after caesarean operation. This may be due to extravasation of fluid after increased capillary permeability, dehydration and inhibition of ADH release due to shock (Detweiller, 1984).

Although, lower blood glucose levels were recorded on post-treatment days in buffaloes that died the difference between survivors and animals that died were not significant (Table). Atwal (1993), however, found significantly lower glucose levels in buffaloes that died after caesarean operation. The decrease in glucose concentrations on subsequent days could have been the result of withdrawal of stress or due to prolonged starvation after obstetrical manipulation leading to exhaustion of glucose pools and glucogenic amino acids.

Analysis of data revealed that buffaloes which died after caesarean section had significantly ($p<0.05$) lower plasma protein levels on post-treatment day 1 than the survivors. Increased adrenal activity in dead animals might have resulted into hypoproteinemia due to increased protein turnover and nitrogen loss (Kaneko, 1989). Moreover, consequent upon treatment, protein requirement for tissue repair may reflect upon protein reserves leading to hypoproteinemia. Loss of blood during surgery with subsequent extraction of interstitial fluid into plasma can also result into low protein levels.

Plasma immunoglobulin concentration was significantly lower at presentation and on post treatment days in buffaloes that died subsequent to obstetrical manipulations as compared to survivors. No report on total plasma immunoglobulins level was available for comparison. Dystocia, a stressful event may depress the immune status of the dam (Yablonskii and Prigara, 1984) thus resulting in poor survival rate.

The increase in values of PCV and Hb and decrease in plasma proteins and immunoglobulins on post-treatment days indicated poor prognosis in the buffaloes that died later on. These changes in blood indices may either be a part of the cause of death or may be a sequelae to metabolic derangements leading to death. It is, therefore, speculated that proper evaluation of animal at presentation regarding anorexia, dehydration, toxaemia and stress is necessary for judicious planning for plasma or blood transfutions to normalise the blood homeostasis.

Table : Haematological and Biochemical indices following obstetrical treatment

Indices	Group	Pre treatment	During treatment	Days post treatment 1	2
PCV (%)	Survivors	34.50 ±1.66	34.25 ±1.32	35.68 ±1.56	32.80 ±1.22
	Dead	34.28 ±2.13	34.43 ±2.33	41.00 ±1.09*C	40.50 2.50*D
Hb (mg%)	Survivors	12.25 ±0.42	12.03 ±0.53	12.30 ±0.16	11.84 ±0.28
	Dead	12.06 ±0.53	12.44 ±0.53	13.28C ±0.63	12.70D ±1.30
Glucose (mmol/L)	Survivors	5.70 ±1.03	5.93 ±0.56	4.77 ±0.60	3.92 ±0.49
	Dead	6.30 ±0.87	6.06 ±0.49	4.49C ±0.78	4.86D 1.14
Proteins (g/dl)	Survivors	7.69 ±0.24	7.60 ±0.20	7.71 ±0.20	7.45 ±0.27
	Dead	7.23 ±0.40	6.97 ±0.18	6.54*C ±0.32	6.85D ±0.30
Immunoglobulins(g/dl)	Survivors	2.45 ±0.08	2.38 ±0.07	2.47 ±0.10	2.38 ±0.08
	Dead	2.03* ±0.12	2.00** ±0.06	1.95**C ±0.03	1.77**D ±0.15

*p<0.05; **p<0.01 Different from survivors. C = Five animals, D = Four animals

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PREVALENCE OF URINARY INFECTION IN BUFFALOES HAVING PROLAPSE OF GENITALIA

S.PRABHAKAR, PARKASH SINGH, G.S. DHALIWAL, A.K. ARORA and G.R.PANGAONKAR

Department of Animal Reproduction,
Gynaecology and Obstetrics
P.A.U., Ludhiana – 141 004.

ABSTRACT

The urine samples from animals (Buffaloes 26 and cattle 11) affected with prolapse of genitalia (both pre & post-partum cervico-vaginal prolapse) collected aseptically, were subjected in in-vitro culture and drug sensitivity to study the presence of bacterial infections in urine, if any, and therapy for the same. Thirty urine samples (81.08%) were positive for bacterial isolates. In majority of buffaloes having urinary infection (n=22). E.coli was the most common organism isolated followed by staph.aureus. Proteus and Klebseiella sp. of organism, whereas in cattle (n=8), E coli and Staph. aureus existed with the same frequency. Gentamycin, Neomycin, Ntrofurandantin and Chloramphanicol were found to be the most sensitive antibiotics against urinary infections in both cattle and buffaloes. It appears that most of the animals with prolapse of genitalia develop urinary infection which, unless properly treated, may delay the response to conventional therapy of prolapse of genitalia.

Prolapse of genitalia in pregnant and post-partum animals is a frequently occurring condition which, if not managed at an appropriate time, may lead to serious complications including abortion, infertility or even death. Success of treatment in such cases depends upon the severity of the condition which may increase from mild to severe due to exposure of the vaginal/cervical tissues to the external environment (Sloss and Dufty, 1980). Many predisposing and causative factors have been proposed for the occurrence of this condition (Nanda, 1979; Verma et.al., 1979 Nanda and Sharma. 1982; Pandit et.al., 1982). Urino-genital Infections (Sharma et.al., 1977) may also be a factor responsible for prolapse of genitalia in cows and buffaloes. The present study was conducted to study the prevalence of bacterial infections in the urine and the most suitable drug in cows and buffaloes having prolapse of genitalia.

MATERIALS AND METHODS

Thirty seven animals (Buffaloes 26 and Cows 11) presented for the treatment of prolapse of genitalia (both pre-and post-partum ,cervico-vaginal prolapse) at P.A.U. Veterinary Clinics were utilised. In the present study, the urine sample were collected aseptically using sterlized urinary cathetar in sterlized test tubes from all the animals before instituting any medical and/or manipulative treatment. The urine samples were then streaked onto sheep blood agar plates and McConkey's lactose agar plates. The

plates were incubated at 37°C for 24-48 hr.The colonies thus appearing on the plates were studied for the cultural, morphological and biochemical characteristics for the identification of the micro-organisms (Carter.1984).

All the bacterial isolates were subjected to in-vitro drug sensitivity through standard . disc-diffusion technique (Bauer et.al., 1966) to find the most sensitive antibiotic against possible urinary infections in such animals.

RESULTS AND DISCUSSION

Thirty (81.08%) urine samples collected from buffaloes and cows having prolapse of genitalia were found to be positive for bacterial isolates. The details of the organisms isolated in the urine samples and the most suitable sensitive antibiotic are presented in the table.

Alout 84.70% buffaloes with genital prolapse had urinary infections. In majority of them (n=11) E.coli was the most commonly occurring organism (n=14). Which was followed by Staph. aureus, Proteus, Klebsiella and Aerobacter sps. Organisms. Likewise in cows having genital prolapse, eight animals (72.7%) were positive for urinary infections wherein E.coli and staph.aureus existed with same frequency. Two other animals had organisms of Proteus and Aerobacter sps. Isolated from their urine.

Sharma et.al., (1977) had opined that the urinary tract infections may induce straining thus, facilitating occurrence of prolapse of genitalia. The high prevalence of urinary infections in the present study substantiated the earlier findings about the importance of urinary tract infections in genital prolapse, as pathogenic bacteria may induce inflammation and denudation of mucosa resulting in tissue damage and release of highly irritating histaminic substances, thus leading to continuous straining. Whether, U.T.I is a cause of genital prolapse or occurs as a sequence to repeated exposure of genital tissue and uretheral orifice can not be dicided from the present investigations, yet, evidence appears to be in favour of latter. No reference could be traced in the available literature to compare the findings. However, presence of urinary infection in more than 80% animals with prolapse of genitalia warrants immediate antibiotic cover for compete cure of such animals.

In-vitro drug sensitivity test carried out with nine antibiotics revealed that Gentamicin, Neomycin and Chloramphenicol were the most sensitive antibiotics against the urinary tract infections in both buffaloes and cows with prolapse of genitalia (Table). All the bacterial isolates were found to be completely resistant to Penicilins with some isolates also resistant to Streptomycin, Co-trimoxazole and Oxytetracycline. It appears important to monitor culture and sensitivity pattern in each animal before adopting any therapeutic approach. Use of urinary antiseptics should also be included in the therapeutic schedule of prolapse of genitalia.

Table

Isolation and in-vitro drug sensitivity of organisms isolated from urine of buffaloes and cows having genital prolapse

Bacteria	Total No.	Sensitive to								
		G	C	N	Nr	O	S	P	Co	Cf
Buffaloes (n=26)										
E.coli	11	9	6	9	5	2	1	-	4	2
Staph.aureaus	5	3	3	4	2	3	2	-	1	-
Proteus sps.	3	3	1	1	2	1	2	-	2	1
Klebsiella sps	2	1	2	2	2	2	-	-	1	-
Aerobacter sps	1	1	1	1	1	-	-	-	-	-
	22	17	13	17	12	8	5	0	8	3
Cows (n=11)										
E .coli	3	3	3	2	3	2	1	-	-	1
Staph.aureaus	3	3	2	2	1	1	1	-	1	2
Proteus sps.	1	1	1	1	1	1	-	-	-	-
Aerobacter sps	1	1	1	1	1	-	1	-	-	-
	8	8	7	6	5	4	3	-	1	3

G-Gentamicin, C-Chloramphenicol, N-Neomycin, Nr.Nitrofurantoin, O-Oxyterracycline, S-Streptomycin, P-Penicilin, Co-Cotrimoxazole, Cf-Cephalexin.

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EFFECT OF POULTRY LITTER FEEDING ON THE REPRODUCTIVE PERFORMANCE OF CROSSBRED HEIFERS

ILA PANDEY, U.K. ATHEYA and S.S. CHAUHAN

Department of Animal Science
G.B.Pant University of Agriculture & Technology
Pantnagar – 263 145 Uttar Pradesh India.

ABSTRACT

Twenty crossbred heifers of 3-6 months of age were distributed into four groups randomly and fed concentrate mixture replaced with 0.25, 37.5 and 50 per cent poultry litter (PL). The heat was detected regularly. After 8 to 10 estrous cycles, animals were inseminated artificially and pregnancy determined by estimating plasma progesterone levels after 18 days and rectal palpation method after 45 days of insemination. It was observed that PL feeding up to 50 per cent level did not have any adverse effect on the age of puberty, estrous cycle length and conception rate of the animals. However, better results were obtained at 37.5 per cent level of PL feeding.

Limited feed and fodder resources in India have necesistated the development of altelrnative non-conventional feed resources. One such waste is poultry litter which can be utilized as an ingredient in ruminant feeding. It is a rich sources of various nutrients such as non-protein nitrogen (NPN), metabolizable energy (ME), amino acids, vitamins and minerals. Crude protein content of PL ranges from 21 to 30 per cent on DM basis. Being a rich source of nutrients, low cost and easy availability, poultry litter is of common use today in India, Many studies showed that there was no adverse effect on growth rate and health of heifers fed with PL up to 40 per cent of concentrate mixute (Reddy et.al., 1990, Kuvera and Salcedo, 1992). Along with growth of animal, regular reproduction is required to achieve the demand for different animal products. The purpose of present study was to determine the effect of PL feeding on the reproductive performance of crossbred heifers by finding out age at puberty, estrous cycle length and of conception rate.

MATERIALS AND METHODS

Twenty crossbred heifers were selected from the herd maintained at Livestock Research Centre of the University. They were distributed into four groups of 5 each. They were fed poultry litter diets from the age of six months till end of the experiment. Level of poultry litter in the concentrate mixture was maintained at 0, 25, 37.5 and 50 per cent for Group. I, II, III and IV, respectively. Poultry litter used for feeding was one year old. Concentrate mixture in group I was composed of mustard cake (30%) ground wheat (33%) deoiled rice bran (34%) mineral mixture (2%) and common salt (1%). In groups II, III & IV the levels of the feed ingrediants were as per NRC requirements (NRC, 1988). The heifers

were also provided with sufficient fresh drinking water.

Heat detection was carried out daily throughout the experimental period by seeing fern pattern of cervicovaginal mucous, standing heat, tone of uterus and by estimating plasma progesterone level (WHO Procedure, 1982). Age at which animal showed first heat was referred as age at puberty. Estrous cycle length was calculated as the days between two consecutive estrous. After observing eight to nine consecutive estrous cycle lengths, the mean was calculated for each heifer. Animals were given prostaglandin i.e Prosolvin (Intercare) 1.0 ml intramuscularly at 11 days apart to synchronize the heat to follow uniform insemination. Pregnancy was determined by estimating plasma progesterone level in the blood serum at 18 days and by rectal palpation method at 45 days after insemination .

The data obtained in this investigation were analysed by using randomized block design and two factorial completely randomized design

RESULTS AND DISCUSSION

Age at Puberty : In the present work, average age of puberty was noted as 612.6 days, 641.4 days, 570.2 days and 670.8 days in I, II, III and IV group, respectively. In all groups the age at maturity was more compared to the reports of Reynolds et.al., (1963) who observed that Zebu cross heifers mature at the age of 460 days. In the present study, the increased age of puberty might because of the absence of opposite sex or bull in the herd (Vsachenoko, 1986). However, age at puberty in all the animals was lower than the age (26 months) reported in Ongole heifers by Venkatramaiah and Rao (1993). The lower age at puberty of experimental heifers may be because the heifers were crossbred and the crossbreeding reduces the age of sexual maturity (Tegegne et.al 1992). The group III had lesser age of puberty as compared to other groups.

Estrous cycle length : The average estrous cycle length of heifers of I, II, III and IV groups were 21.76, 21.10, 20.48 and 20.40 days, respectivley. Estrus cycle length of all the heifers laid within the range of 15-25 days. This range was approximately similar to the range reported by Holroyd et.al., (1988) who found that when nutrition was maintained at normal level the cycle length was 18.24 days in ½ Brahman heifers .

On statistical analysis there was no significant different in the age at puberty and estrous cycle length between the heifers of treatement groups and control group.

Conception rate : Out of twenty heifers inseminated, only eight could be pregnant. The lowered conception rate of animals might be due to the higher ambient temperature (31.5-43.3^{o}C) during May-June. It was in agreement with the findings of Barker et.al., (1990) In present study the conception rate was 40% 20% 60% and 40% in Group I, II, III and IV respectively.

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CONCENTRATION OF SERUM THYROID HORMONES DURING PREGNANCY PARTURITION, POSTPARTUM & LACTATION IN MADRAS RED SHEEP

S. ESWARI, S. VISWANATHAN, V. LEELA, MD.NAYEEM and K GAJENDRAN*

Department of Physiology & Biochemistry
Madras Veterinary College
Chennai 600 007.

ABSTRACT

Effect of pregnancy, parturition, postpartum and lactation on Thyroid secretion rate were studied in 15 Madras Red ewes. Blood samples were collected on the day of mating; and on 2nd 3rd, 4th and 5th month of pregnancy and on day of parturition, 1,2,5,10,15 and 20 days of postpartum, early, mid, late lactation period and during dry period. The serum sample was separated out for estimation of thyroid hormones viz, Thyroxine, Triiodothyronine and free thyroxine. During pregnancy the thyroid hormones increased significantly from the day of mating and reached the highest level during 4th month of pregnancy and decreased significantly to the lowest level on the day of parturition and there after increased significantly during postpartum period. The circulating thyroid hormones were significantly lower during lactation period and reached the normal level during dry period.

Thyroid hormones control the metabolic process, growth differentiation, reproduction and lactation. During pregnancy and parturition, the thyroid hormone level oscillates (Duckes, 1995). The expulsion of foetus from the mother at parturition resets the endocrine status in the mother. After parturition the dam is relieved of the stress of young one and metabolic and physiological alteration takes place. Riis and Madsen (1985) have demonstrated the thyroid hormones with the stage of lactation in goats. As the literature available on the changes in the thyroid hormone levels during pregnancy, parturition, postpartum and lactation in sheep are scanty, a study was made to evaluate the levels of these hormone during the above period.

MATERIALS AND METHODS

Animals maintained at Livestock Research Station, Kattupakkam were the subject of this study. Blood samples were collected from 15 Madras Red ewes by jugular vein puncture on the day of mating. 2nd 3rd, 4th, 5th, months of pregnancy, on the day of parturition and on 1,2,5,10,15 and 20 days of postpartum. To study the thyroid hormones level during lactation, the lactation period was divided into three phases. i.e., early (0-2 weeks), mid (3-6 weeks) and late (11-14 weeks) lactation and for control, blood samples were also collected during dry period. Clear serum was separated out by centrifugation at 3000 rpm for 20 minutes and stored at –20° C for

* Professor and Head, Livestock Research Station, Kattupakkam.

hormone assay. Serum T_4 and T_3 were quantified by RIA technique described by Abraham (1977) and Chopra (1971) using RIA kits supplied by Bhaba Atomic Research Centre, Mumbai and Serum fT_4 was estimated by RIA technique by Anderson et.al., (1988) using coat – A – count fT_4 kit supplied by Diagnostic Product Corporation. Los Angels. Statistical analysis were made as per Snedecor & Cochron (1967).

RESULTS AND DISCUSSION

The levels of thyroxine (T_4). Triiodo thyronine (T_3) and free thyroxine (fT_4) during different stages of pregnancy, parturition, post partum and during lactation is shown in the Table. The T_4 level on the day of mating was 6.18 ± 0.11 µg/dl which agreed with the values of Okab et.al., (1993) in Barki ewes and Sutherland and Irvine (1974) in sheep. The T4 values showed a nonsignificant increase during 2nd , 3rd , month and significant increase ($p<0.01$) during 4th month. The level declined on 5th month and the lowest value was recorded on the day of parturition. Annison and Lewis (1959) recorded a nonsignificant decrease in plasma T_4 concentration during pregnancy. Prasad (1990) has recorded highest levels of T_4 during 3rd month of pregnancy in ewes. The increase in the levels during gestation may be due to the demands from growing foetus. The decline in the T_4 levels during late pregnancy and on the day of parturition was attributed to the alterations in the maternal body function during pregnancy. Nathanielsz (1973) postulated a negative feed back control by the active foetal thyroid as a probable cause of reduction in maternal T_4. Dalvi et.al., (1990) have stated that during late pregnancy, the blood volume and cardiac output increases with the concomitant dilution of blood biochemicals including T_4 in dairy cows.

The serum T_3 levels revealed a significantly higher values during 2nd, 3rd and 4th month of pregnancy as compared to the values on the day of mating. The values on 5th month was significantly lower ($p\leq0.01$) than previous months but higher than the values on the day of mating. The lowest T_3 value was recorded on the day of parturition. Obkab et.al., (1993) have reported a lower T_3 concentration in Barki and Rahmani ewes as compared to the present study and the mean T_3 concentration did not vary significantly during different stages of pregnancy. Bhattacharya et.al., (1994) have found nonsignificant increase in T_3 level during pregnancy in goats. The rise in T_3 level during late pregnancy was attributed to increase in estrogen secretion in buffaloes (Rao et.al., 1978). Klein et. al., (1978) have studied the effect of glucocorticoids during later stages of pregnancy and found that the rise in this hormone produced an inhibitory effect on the pituitary thyroid axis.

The fT_4 levels recorded highest value on 4th month of pregnancy ($p\leq0.01$) and lower value on the day of parturition ($p\leq0.01$). There was no difference noticed among other periods. The trend in changes in concentration of fT_4 in similar to the changes observed in T_4 and T_3. As

there are no earlier reports in the literature available, the values in the present investigation could not be compared. The changes in the fT_4 values indicated that fT_4 value is dependant on total thyroxine level as fT_4 is getting released from the Thyroid Binding Globulin (TBG.)

The T_4 values on day of parturition and on 1 and 2 days after parturition did not vary, whereas the value on 5 and 10 days of post partum were higher and the highest values was observed on 20 days of post partum. Similar results were observed by Heitzman and Mallinson (1972) in cows. The low T_4 profile during first few days of post partum might be due to the utilization of T_4 for mammary development and lactogenesis. The T_3 values also exhibited a similar trend like that of T_4. Vanjanock and Johnson (1975) have expressed that more thyroid hormones are excreted through mammary gland cows. The fT_4 values were significantly lower ($p \leq 0.01$) on the day of parturition and on day 1 as compared to other days of post partum. There was a nonsignificant steady increase in fT_4 levels from 2 to 20 days after parturition. It is obvious that the fT_4 level is a true indicator of the thyroid activity and as the lactation stabilizes after few days the values did not vary significantly.

Table :Levels of Thyroxine Triiodothyronine and free Thyroxine during pregnancy, parturition and lactation in Madras Red sheep.

Period	Thyroxine µg/dl	Triiodothyronine ng/ml	Free Thyroxine ng/dl
Day of mating	$6.18^{a} \pm 0.11$	$0.78^{b} \pm 0.04$	$0.92^{b} \pm 0.2$
During Pregnancy			
2nd month	$6.26^{a} \pm 0.32$	$1.41^{a} \pm 0.05$	$0.93^{b} \pm 0.04$
3rd month	$6.49^{a} \pm 0.25$	$1.47^{a} \pm 0.06$	$1.00^{b} \pm 0.13$
4th month	$7.72^{b} \pm 0.35$	$1.52^{a} \pm 0.06$	$1.35^{a} \pm 0.14$
5th month	$5.92^{a} \pm 0.14$	$1.10^{c} \pm 0.04$	$0.72^{bc} \pm 0.06$
Day of parturition	$3.66^{c} \pm 0.21$	$0.33^{d} \pm 0.01$	$0.54^{c} \pm 0.03$
During post-partum			
1st day	$4.13^{a} \pm 0.04$	$0.73^{b} \pm 0.02$	$0.67^{b} \pm 0.05$
2nd day	$4.06^{a} \pm 0.22$	$0.69^{b} \pm 0.03$	$1.04^{a} \pm 0.08$
5th day	$5.53^{bc} \pm 0.14$	$0.72^{b} \pm 0.01$	$1.21^{a} \pm 0.09$
10th day	$5.97^{bc} \pm 0.10$	$0.86^{c} \pm 0.03$	$1.06^{a} \pm 0.06$
15th day	$5.31^{b} \pm 0.11$	$0.88^{c} \pm 0.02$	$1.26^{a} \pm 0.16$
20th day	$6.01^{c} \pm 0.10$	$0.89^{c} \pm 0.03$	$1.31^{a} \pm 0.16$
Lactation period			
Early	$4.06^{c} \pm 0.22$	$0.67^{c} \pm 0.02$	$0.86^{a} \pm 0.01$
Mid	$5.18^{a} \pm 0.20$	$1.02^{ab} \pm 0.07$	$1.07^{b} \pm 0.04$
Late	$5.31^{a} \pm 0.11$	$1.06^{a} \pm 0.04$	$1.04^{ab} \pm 0.04$
Dry period	$6.04^{b} \pm 0.10$	$0.88^{b} \pm 0.02$	$1.41^{c} \pm 0.13$

Means having atleast one common superscript do not differ at 5% level.

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REPRODUCTIVE PERFORMANCE OF ONGOLE CATTLE

K. BABURAO[1] and K. SADASIVA RAO[2]

Cattle Project,
Lamfarm, Guntur – 34 Andhra Pradesh

ABSTRACT

The records of 215 Ongole cattle of over a period of 12 years were analysed to study their reproductive performance. The Important reproductive parameters recorded were age at puberty, 744.86 ± 11.06 days ; body wt. at puberty, 257.05 ± 2.65 kgs; age at first calving 1063. 70 ± 11.70 days; post partum service period in cows 113.35 ± 2.02; number services per conception 1.53 ± 0.03 and intercalving period in cows 407.79 ± 2.38 days. The study showed that the reproductive performance of ongole cattle was on par with milch breeds and good when compared to other Indian breeds.

The Ongole cattle is one of the recognised breeds of India and also famous in foreign countries for its hardiness, adoptability and disease resistence. This is a dual purpose breed used for draught purpose and milk production. The information on reproductive performance of cattle will be very valuable for improvement of livestock as these traits affects life time production of the animal and reduces unproductive period. Hence this present study was under taken to report the reproductive performance of Ongole cattle.

1. Research Officer, Animal Reproduction
2. Associate Professor, College of Veterinary Science Rajendranagar, Hyderabad

MATERIALS AND METHODS

The data pertaining to the records of 215 Ongole cattle were subjected to statistical analysis as per the methods of Snedecor and Cochran (1967) to study their reproductive performance. The data was collected from the reproductive records of Ongole cattle maintained at Cattle Project, Lam Farm, Guntur over a period of 12 years from 1986 to 1997. The data on the animals which have reproductive problem were excluded while calculating the service period and the animals which gave still births and abortions were not included for calculating the intercalving period.

RESULTS AND DISCUSSION

Age and weight at puberty: The average age at puberty in the present study was 744.86 ± 11.06 days, which was closer to the Ongole crossbreds of different exotic levels (Babu Rao et.al., 1984) and lower than the other Indian breeds (Joshi and Phillips, 1953). The average body weight at puberty was 257.05 ± 11.54 Kgs, similar observation was recorded in Ongole crossbreds (Babu Rao. et.al., 1984).

Age at first conception : The average age at first conception was 780.57 ± 11.54 days, which was closer to the Ongole crossbreds (Babu Rao et.al.,

1984) and lower than other Indian breeds. (Velhankar, 1973).

Number of services per conception: The number of services required per conception in heifers was 1.52 ± 0.05 and in cows 1.53 ± 0.03 and the difference between them was non significant, similar observation was reported in Ongole crossbreds having mixed exotic blood of Jersey and Freisian. (Babu Rao et.al., 1984).

Age at first calving: The average age at first calving observed in the present study was 1063.70 ± 11.70 days, this observation was lower than the reports made by Joshi and Phillips, (1953) and Venkayya and Anantha Krishna (1956) in other Indian breeds, Krishna Rao (1966) and Rama Mohan Rao, et.al., (1969) in Ongole cattle. Similar observation was reported in Ongole crossbreds by Babu Rao et.al., (1984) and Venkateswarlu (1971) in Ongole cattle.

Gestation period: The average length of gestation period was 287.24 ± 0.28 and 289.22 ± 0.41 days respectively in heifers and cows and the difference between them was significant ($p \leq 0.05$). Similar gestation length was reported in Ongole cattle by Rao and Taylor (1971) and Rajulu and Rama Mohan Rao (1966).

Postpartum service period: The average first postpartum service period and subsequent postpartum service periods were 118.54 ± 4.49 and 113.35 ± 2.02 days respectively and the difference between them was nonsignificant. The present observation was very much lower than the reports of Krishna Rao (1966) and Venkateswarlu (1971) in Ongole cattle and Babu Rao et.al., (1984) in Ongole crossbreds.

Intercalving period: The average length of first calving interval and subsequent calving intervals were 442.14 ± 7.54 and 407.79 ± 2.38 days respectively and the difference was highly significant ($p \leq 0.01$). The present observation was much lower than the reports published by Krishna Rao (1966) Rama Mohan Rao et.al., (1969) and Sarma (1981) in Ongole cattle and other Indian breeds of cattle by Venkayya and Anantha Krishna (1956). The longer calving interval in first calvers in the present study might be due to lactational stress and early maturity of the animals.

The observations made in the present study indicates that the reproductive performance of Ongole cattle was on par with milch breeds and corssbred cattle and good when compared to other Indian breeds.

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STUDY ON PELVIC DIMENSIONS IN EUTOCIC CROSSBRED JERSEY COWS

S. SELVARAJU [1], P. SELVARAJ [2], D. RAJENDRAN [3], and L.N. PURBEY[4]

Division of Animal Reproduction
Indian Veterinary Research institute
Izatnagar Uttar Pradesh

ABSTRACT

Eighty five Jersey crossbred cows that had not encountered any parturient difficulties were selected and grouped according to their parity. In all the five groups external pelvic dimensions were measured 3 months after calving. The transverse pelvic inlet and outlet increased upto third calving. The fist calving group had significant difference ($p<0.01$) with other groups. The vertical pelvic diameter increases after every parturition and the vertical pelvic inlet in first calving group had significant difference with 5th group whereas in vertical pelvic outlet had no significant difference between groups.

Dystocia is the commonest cause (over 50 per cent) of perinatal mortality of calves (Bellows et.al., 1984). Major cause of dystocia is disproportionately large calf size at birth in relation to pelvic area. Application of pelvimetry is used by bovine practioners in an attempt to reduce dystocia due to fetomaternal disproportion (Price and Wiltbank, 1979). The study of the pelvimetry is essential particularly where selective breeding or crossbreeding is practiced. One can form an idea about the shape and size of pelvis and thus about pelvic canal based on the relationship between different pelvic diameters, which will be useful for taking measures to avert the occurrence of dystocia.

MATERIALS AND METHODS

The present study was carried out in 85 crossbred cows maintained in a private dairy farms at Erode district, Tamil Nadu state, which did not have any dystocia problems. All those cows were maintained on uniform ration and under scientific management system.

The experimental cows were divided into five groups according to their parity as follows.

Group I (n=43) : First calvers aged between 2½ and 6 years.
Group II (n=10) : Second calvers aged between 4 and 6 years.
Group III (n=6) : Third calvers aged between 6 and 9 years.
Group IV (n=9) : Fourth calvers aged between 7 and 10 years.
Group V (n=11) : Fifth and above calvers aged between 8 and 14 years.

In all the groups, the external pelvimetry was taken at about three months after calving. The measurements of the external pelvic

1. Ph.D. Scholar.
2. M.V.Sc. Scholar
3. M.V.Sc. Scholar, Division of animal Nutrition.
4. Head.

dimensions were taken as described by Craig (1930).

1. The distance between the two angles of the haunch (ilium) was measured by using a straight scale placed vertically against each haunch and the space between them was measured. (a).

2. The distance between the two ischial tuberosities was taken directly with a measuring scale (b).

3. The height from the hip-joint to the level of the highest point of the croup was measured by placing a straight scale horizontally across the summit of the croup, while another was laid in the same direction along the trochanter and ischial tuberosity, the vertical distance between the two scales was measured (c). Based on the above measurements the following diameters of the pelvic outlet and pelvic inlet were calculated.

i. Transverse diameter of the pelvic outlet.

$$(x) = \frac{1}{4}(a+b)$$

II. Vetical diameter of the pelvic outlet (superoinferior diameter)

$$(y) = \frac{3}{4}c$$

III. Transverse diameter of the pelvic inlet

$$= \frac{12.2}{10} X$$

IV. Vertical diameter of the pelvic inlet

$$= \frac{13}{10} Y$$

The data were analysed statistically as described by Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

The mean values of pelvic dimensions of external and internal pelvimetry were pressented in Table. The distance between the angle of ilium was significantly more ($p<0.01$) in the second and above calvers than the first calvers. The distance between the ischial tuberosity in second calvers also increased significantly ($P<0.01$), however no significant difference ($p<0.05$) with fourth and fifth calvers. The distance between the sumit of group and hip joint showed no significant difference between all these five groups.

The mean transverse pelvic diameter was lower than the mean value (17.75 cm) reported by Craig (1930). However these values fall within the range of 16.88 to 19.38 cm reported by various authors (Craig,1930 and Roberts, 1971). The mean transverse pelvic outlet diameter was also lower than the value 18.75 and 22.5 cm reported by Craig (1930). However the range values fall within the lower margin of the value 14.6 to 19.00 cm obtained by Roberts (1971). Mean transverse pelvic dimensions (both pelvic inlet and outlet) show increasing trend upto 3rd calving after that, decreased. However, the transverse pelvic diameter in first calving had a significant difference ($P<0.01$) with other groups. The decrease in the transverse pelvic diameter after 3rd calving may be due to action of various hormones during

parturition and subsequent effect on the maternal pelvis. Further, increase in weight of the quadruped animal may increase vertical pelvic diameter and also decrease transverse pelvic diameter. Senility may also reduced the transverse pelvic diameter after third calving. However, this needs to be confirmed in animals.

The mean vertical pelvic inlet diameter was lower than the reported value of 19.75 (Craig, 1930) and 19.0 to 24.1 cm (Roberts, 1971). The mean vertical pelvic outlet diameter was also lower than the reported value of Craig (1930). The vertical pelvic diameter increases gradually in every parturition. The vertical pelvic inlet diameter in first calvers had a significant difference ($p<0.01$) with fifth and above calvers group only. The vertical pelvic outlet had no significant difference ($p>0.05$) between different groups.

The mechanism of pelvic canal expansion in cattle is unknown. Musah et.al.,_(1986) suggested that increase in pelvic dimension in beifers may result not only from the relaxation of pelvic ligaments but also from a remodelling of the public symphysis.

The values obtained in the present study falls within the lower margin of the exotic breeds. The samples were collected from an animals of non-homogenous nature which were subjected to different factors such as genetic, nutritional, managemental etc., which are responsible for shaping the pelvis. However, detailed study on the large size of sample will be still helpful in arriving at average pelvic dimensions, so that we can reduce the calf mortality by the way of selection.

Table : Dimensions of Pelvimetry (MEAN ± SE cm)

Party	(a)	(b)	(c)	Transverse pelvic diameter		Vertical pelvic diameter	
				Inlet	Outlet	Inlet	Outlet
1st Calvers (n=43)	34.20 ± 0.05 ^{d}	13.94 ± 0.03 ^{d}	18.17 ± 0.03	14.57 ± 0.15 ^{d}	11.97 ± 0.18 ^{d}	17.65 ± 0.03 ^{d}	13.62 ± 0.02
2nd Calvers (n=16)	36.78 ± 0.08 ^{e}	15.50 ± 0.08 ^{e}	18.50 ± 0.06	15.97 ± 0.18 ^{e}	13.08 ± 0.04 ^{e}	18.03 ± 0.06 ^{de}	13.80 ± 0.54
3rd Calvers (n=6)	38.00 ± 0.24 ^{e}	16.00 ± 0.26 ^{e}	18.83 ± 0.41	16.47 ± 0.31 ^{e}	13.50 ± 0.10 ^{e}	18.36 ± 0.04 ^{de}	14.13 ± 0.31
4th Calvers (n=9)	38.72 ± 0.32 ^{e}	14.67 ± 0.20 ^{de}	19.56 ± 0.15	16.29 ± 0.45 ^{e}	13.35 ± 0.12 ^{e}	18.73 ± 0.11 ^{de}	14.67 ± 0.01
5th and above Calvers (n=11)	38.23 ± 0.25 ^{e}	15.05 ± 0.11 ^{de}	19.86 ± 0.14	16.22 ± 0.33 ^{e}	13.32 ± 0.02 ^{e}	19.31 ± 0.14 ^{e}	14.90 ± 0.11

Distance between (a) angle of ilium; (b) ischial tuberosity and (c) between croup and hipjoint

Same Column bearing superscript d, e differ significantly ($P<0.01$)

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HAEMATOLOGICAL AND BIOCHEMICAL CHANGES IN PERIPARTURIENT FEMALE CAMELS (Camelus dromedarius) *

A.M. PANDE**, S.P. AGARWAL, A..K. RAI and N.D. KHANNA

Department of Veterinary Physiology. C.C.S. Haryana
Agricultural University, Hisar – 125 004.

ABSTRACT

Blood samples from eight pregnant single humped camels (Camelus dromedarius) were collected during one month pre-partum and two months post-partum period. Haematological attributes studied were RBC,WBC, PCV, Hb, MCV, MCH and ESR and the biochemical constituents included glucose, total proteins, cholesterol and ascorbic acid. The data did not reveal significant differences in the values of haematological attributes except for WBC which was significantly higher around parturition and then declined by about one month post-partum. Among biochemical constituents, the mean glucose concentration elevated around parturition while that of cholesterol during later stages of post-partum period. The total proteins and ascorbic acid did not exhibit significant changes during pre and post-partum stages.

The information on haematological and biochemical aspect of female camels during periparturient period is meager (Elias and Yagil, 1984; Dahiborn et.al., 1992). Therefore, an effort was made to monitor these changes in female camels during one month before and two months after parturition.

* Part of Ph.D.thesis of first author to C.C.S. Haryana Agricultural University, Hisar-125004

** Corresponding author:- Present Address : - Associate Professor, Vety. Physiology, college of Vety. Sci. & A.H. Sardar Krushinagar – 385 506.

MATERIALS AND METHODS

Eight single humped healthy pregnant she-camels (Camelus dromedarius) in the last month of gestation were used for this study. The animals belonged to National Research Centre on camel, Bikaner and were kept under standard management condition. Blood samples from these animals were collected by jugular venipuncture on days 28, 21, 14, 7, 5, 3,1 pre-partum and 1,3, 6, 13, 20, 27, 34, 41, 48, 55 and 62 post-partum in one set of heparinized tubes to study haematological parameters and for removal of plasma and in another set of tubes for separation of serum. The whole blood was used for haematological parameters. Whereas plasma and serum samples were utilised for the analysis of biochemical constituents.

Haematological constituents viz., RBC, WBC, MCV and PCV were determined using Coulter Counter model ZF and haemoglobin concentration was estimated in Haemoglobinometer from Coulter Electronics Ltd., England.

The blood biochemical constituents viz., glucose, cholesterol and total proteins were determined in serum at 37^{o}C. in Beckman DV clinical

automated analyzer by using Lupin reagent kits for glucose and cholesterol and Ortho reagent kits for total proteins. Ascorbic acid in blood plasma was determined by 2, 6 dichlorophenol indophenol Dye method as described by Bauer et.al., (1974). The data were statistically analysed for stage to stage difference using Student's test in ECIL micro 32 computer.

RESULTS AND DISCUSSION

During pre-partum and post-partum periods the RBC, WBC, PCV and Hb ranged between 7.05 ± 0.31 to 8.23 ± 0.39 10^6/ mm^3 ; 6.30 ± 0.26 to 10.40 ± 0.88 10^3/mm^3 ; 21.25 ± 1.28 to 26.03 ± 1.70 % and 10.28 ± 0.39 to 11.72 ± 0.61 g/dl respectively. Derived attributes of RBC and Hb also remained almost constant during pre and post partum period.

Although certain fluctuations were observed in some of these parameters, stage to stage variations were not significant statistically, except for WBC which were significantly higher during one week preceeding and three weeks following parturition. Higher leukocyte count at and around parturition has also been reported by Elias and Yagil (1984). A little higher leukocyte number in peripartum camels could be due to stress of impending parturition and high cortisol levels (Agarwal et.al., 1992) which have a marked effect on the number and the proportion of different types of leukocytes, particularly the lymphocytes and the neutrophils. It appears that camel RBC is fairly resistant to physiological trauma of pregnancy and parturition manifesting only marginal changes in its number and haemoglobin contents. The ESR in camel is extremely low (Bokori, 1974). A value of 1 or less than 1 mm /hr was encountered in this study which is in confirmity with the results of Elias and Yagil (1984) In camel. It has been reported that, the relative proportion of plasma proteins rather than their absolute concentration determine the ESR and high albumin percentage in the plasma causes retardation of sedimentation rate (Dukes, 1984). Since camel plasma has relatively higher albumin concentration (Yagil, 1985), it may be one of the contributory factors for the low sedimentation rate in camel.

The levels of glucose obtained in this study (78.85 ± 6.36 to 103.81 ± 3.94 mg/dl) are within the range reported in camels by other workers (Soliman and Shaker, 1967; Al-Ali et.al., 1988). It was observed that glucose levels peaked around the day of parturition (103.81 ± 3.94 mg/dl) with relatively lower values during pre (79.04 ± 4.91 to 100.00 ± 5.20 mg/dl) and post partum (78.05 ± 6.36 to 88.09 ± 6.63 mg/dl period. Similarly, lower glucose levels during post-partm period in camel recorded by Dahlborn et.al., (1992) and Elias and Yagil (1984) was adduced to excessive need of gluopse for lactose synthesis in the udder. The chol esterol concentration (24.70 ± 2.40 to 40.44 ± 3.66 mg/dl) was on the lower side during the pre-partum period but elevated steadily after parturition for about a month and then became stable. The changes in cholesterol seem to be associated with fat metabolism which is substantially altered to sustain the stress of

parturition and for synthesis of milk following parturition. The total blood protein (5.31 ± 0.16 to 5.89 ± 0.21 mg/dl) and ascorbic acid (2.88 ± 0.52 to 5.15 to 0.40 mg/dl) levels were fairly constant during pre and post partum stages except for a little but nonsignificant higher value of ascorbic acid on day before parturition (5.15 ± 0.40 mg/dl) which may be of ovarian origin. Overall it seems that camel tends to maintain its blood composition to a greater extent than other domestic animals under stressful stimuli of physiological nature.

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ANDROLOGICAL EVALUATION OF YAK BULL–I CORRELATION BETWEEN AGE, BODY WEIGHT, SCROTAL AND BODY MEASUREMENTS.

T.K. MOHANTY[1], M.R. ANSARI[2], R.N. PAL[3], M.SARKAR[1], D.B. MONDAL[1], S.K. BARARI[4], and U.N. NANDANKAR[4]

National Research Centre On Yark (ICAR)
Dirang-790 101. West Kameng District
Arunachal Pradesh, India.

ABSTRACT

The relationship among body measurements, age and body weight were studied in 6 adult yaks bull aged 5 to 6 years. Age was significantly correlated with heart girth and height at back, body weight. Body weight was significantly correlated with height at wither, back girth at heart and belly. Scrotal circumference was significantly correlated with height at wither and punch girth. Scrotal diameter is significantly correlated with height at wither and punch girth. Age was having greater effect on the scrotal circumference than that of body weight. Conversely body weight was having greater positively correlation on the scrotal diameter than age of the yak (r=0.65 vsr = 0.58). Scrotal diameter was also positively correlated with scrotal circumference. The maximum scrotal length was 27cm with an average of 26.25 ± 1.25 cm. Sexual maturity in yak bull was observed at around 3-4 years of age in the fourth breeding season, when the average body weight of the male was 170.33 ± 8.6 kg and the S.C. was 18.0 ±1.8 cm.

Yak (Poephagus grunniens L.) is a large multipurpose bovid of the high altitude (3000 m to 6000 m above mean sea level) which provide milk, meat, hair, wool, transport, draught and fuel to the highlanders. It has the inherent capacity to withstand low temperature and oxygen environment of the high altitude (Pal. 1993).

The selection of bull for breeding purpose is generally done by traditional method of selecting the bull mostly according to phenotypic character from the same herd. However, there is natural selection due to fighting for dominance among the bulls for achieving the mating position, Natural mating is only possible and practised in yak.

Breeding soundness evaluation of bull in the natural mating conditions are used to assess the reproductive potential of the bull. Scrotal measurements have been extensively used in cattle (Chenoweth and Ball, 1980, Coulter and Keller, 1982 and Das and Tomer, 1995), ram (Lino 1972), goat (Bongso et.al., 1982), buffalo Nema and Kodagali, 1994, Das and Tomer, 1995) and swamp buffalo (Bongso et.al., 1984) for breeding soundness evaluation which is lacking in yak. Further SC along with its consequences is highly heritable.

1.Scientist, NRC-Yak. 2. Senior Scientist, A.R.Division IVRI Izatnagar. 3. Emeritus Scientist, NRC – Yak. 4. Farm Manager, NRC-Yak

The objective of the present study is to generate basic informations on the relationship of age, body and scrotal measurements in young and adult bulls which later on will be used for selection of breeding bull on the field condition by the yak herdsman for selecting their bull for natural mating.

MATERIALS AND METHODS

Twenty male yaks ageing between 36 to 60 months and weighing between 150 to 300 kg were used in this study. Body measurements and scrotal measurements were taken in the standard proforma in monthly basis for two breeding season (May-Oct) in three groups of Yak (≤4, ≤5 and ≤6 yrs) for prediction of sexual maturity and growth, Data was analysed with standard statistical methods and the correlation between age, body weight body measurements and scrotal measurements in bulls was calculated by regression analysis (Snedecor and Cochran, 1971)

RESULTS AND DISCUSSION

The body weight, scrotal circumference, scrotal diameter and hanging length of the scrotum for three groups of male yaks are presented in table.1. The maximum scrotal length was 27 cm with an average of 26.25 ± 1.25 cm. Sexual maturity in yak bull was observed at around 3-4 years of age in the fourth breeding season, when the average body weight of the male was 179.33 ± 8.6 kg and the S.C was 18.0 ± 1.8 cm. Scrotal circumference increased markedly between 4 to 5 years of age, whereas, body weight increased significantly between 3 to 4 years of age. Scrotal diameter at 3 years of age was 7.58 ± 1.45 cm, which increased steadily to 9.5 ± 0.46 cm at 5 years of age. Similarly, scrotal hanging length increased proportionately from 13.17 ± 1.75 to 18.0 ± 1.9 cm with age and testis size. The age, body weight and body measurements of six adult yak bulls are presented in table.2 The body measurements of adult yak bulls indicate about the square conformation with big belly, short and strong foot.

Correlation between age, body weight and body and scrotal measurement revealed scrotal measurement were positively correlated (r=0.56, 0.49 & 0.56, 0.58) with age and body weight. Age was having greater effect on the scrotal circumference than that of body weight. Conversely body weight was having greater positive correlation on the scrotal diameter than age of the yak (r=0.63 vsr =0.58) Scrotal diameter was also positive correlated with SC and SD was highly correlated with the height at wither, age was correlated with height at back and body length was highly correlated with age.

In winter yak in the natural pasture lost 20 to 30 per cent of their body weight due to incliment weather, scanty food resource and again regain the lost weight in the next summer in the alpine pasture, which delay in attaining the required body weight for sexual maturity. Scrotal measurements were positively correlated with age and body weight in growing bulls, which is similar to the reports of other workers

(Boundron and Brinks, 1986 Coulter and Foot, 1979 and Nema and Kodagali, 1994) in cattle. The testicular size in the present observation increased more rapidly in the young bull and gradually in the mature bull, which is in agreement with the findings by Coulter et.al., (1975) in cattle and by Kodagali and Doshi, (1996) in buffalo. Body weight was having greater positive correlation on the scrotal diameter of yak than age, which was also true in cattle (Wildeus, 1993). Age was having greater significant correlation with heart girth and height at wither in yak, which was reported in cattle by Mohanty, et.al., (1991) in Holstein Friesian bull. Although the scrotal hanging length is less in Yak which have reached sexual maturity never able to retract the testicle completely close in the body, however, immature bull below three years of age have the capacity to do the same in winter. In the high altitude animal due to very low temperature, the size of the testes are small with a very strong cremaster muscle, which is an adaptation to keep the scrotal temperature optimum for sperm production in cold environment (Li and Weiner,1995).

Table.1 Body Weight (kg) and Scrotal Measurements (cm.) in Different Age Groups in Yak. (Mean ± S.D, Range)

Groups	N	Body Weight	Scrotal Circum fenence	Scrotal Diameter	Scrotal Hanging length
≤ 6 years	6	268.08 ± 15.24 (257-298.5)	26.25 ± 1.25 (25-27)	9.5 ± 0.46 (9.1-10.2)	17.00 ± 1.9 (15-19)
≤ 5 years	8	230.18 ± 16.94 (216-154)	21.78 ± 2.04 (17.5-23.5)	8. 9 ± 0.76 (7.65-9.75)	14.63 ± 2.14 (12-17)
≤ 4 years	6	179.00 ± 8.6 (170-187)	18.0 ± 2.04 (16-19.5)	7.58 ± 0.45 (7.15-8.0)	13.17 ± 1.75 (11.5-15.0)

Table –2 Average age, Body Weight and Body Measurements in Adult Male Yak. (Mean ± S.D.Range, N=6)

1.	Age	61.33 ± 2.06 (60 - 64) months
2.	Body Weight	268.08 ± 15.24 (257- 295) kg
3.	Body Length	176.7 ± 4.32 (170 -180) cm
4.	Shoulder to Buttock angle distance	128.67 ± 2.42 (124 -130) cm
5.	Shoulder to Pin angle distance	130.34 ± 4.2 (125 –132) cm
6.	Height at wither	119.17 ± 2.79 (117 – 124) cm
7.	Height behind wither	112.17 ± 1.94 (110 – 115) cm
8.	Height at back	111.17 ± 3.19 (106 – 115) cm
9.	Height at Sacrum	112.34 ± 4.03 (106 – 188) cm
10.	Heart Girth	164.5 ± 3.73 (159 – 170) cm
11.	Belly Girth	185.34 ± 9.33 (169 – 196) cm
12.	Punch Girth	150.67 ± 9.44 (144 – 164) cm

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ULTRASTRUCTURAL CHANGES IN SPERMS AFTER TESTICULAR DEGENERATION IN BUCKS*

D. ANTOINE** and S.R PATTABIRAMAN

Department of Obstetrics and Gynaecology
Madras Veterinary College
Chennai 600 007.

ABSTRACT

Six adult tellicherry bucks were subjected to induced testicular degeneration by scrotal insulation. Semen collected artificially were subjected to ultrastructural studies. Sperm head showed three stages of ultrastructural changes. In stage one changes confined to plasma membrane, stage two to acrosome, stage three to mucleus. Further accumulation of membrane bound vesicles. vacuoles and fibrous structure close to sperm head were observed. In the main piece, mitochondrial swelling, axonemal disorganization hair pin curved deformity were also noticed.

The present investigation was taken up to study the effect of scrotal insulation induced testicular degeneration in bucks on ultrastructural changes in sperms.

MATERIALS AND METHODS

Six tellicherry bucks aged 3 years were subjected to scrotal insulation with scrotal bag for 48hrs. Semen collection was made with the help of an artificial vagin. The semen collected during the first three weeks starting from day 4 after scrotal insulation were subjected to electron microscopic studies.

The gluteraldehyde fixed sperm pellets were washed three times in phosphate buffer PH 7.4 and post-fixed in 1 per cent osmium tetraoxide at 4^0 C for 12 hrs. They were then dehydrated in graded acetone and embedded in spurr low viscosity embedding resin. (Bio-Rad, Microscience Division, USA) one micron thick sections were mounted on glass slides and stained with basic toludine blue for screening under light microscope. Ultra thin sections prepared using Raichert Jung ultracut microtone with diamond knife were collected on copper grids, stained with uranyl acetate and lead citrate and were then examined in Joel electron microscope, Photographs were taken at different magnifications.

RESULTS AND DISCUSSION

Ultrastructural changes in the sperm head were clearly in three stages. In stage one changes were confined to plasma membrane. (Fig) In stage two to acrosome and in stage three to sperm nucleus. Williamson (1974) also reported similar changes in rams after scrotal insulation. During the first week post scrotal insulation the plasma membrane was closely applied over the acrosome, the sub-acrosomal space was prominent. A dark electron dense band like area at the marginal thickening of the acrosome and the

- Part of the Ph.D Thesis submitted by first author in Tamilnadu Veterinary and Animal Science University Chennai.

** Associate Professor, Department of Obstetric.and Gynaecology, Rajiv Gandhi College of Veterinary & Animal Science , Pondicherry.

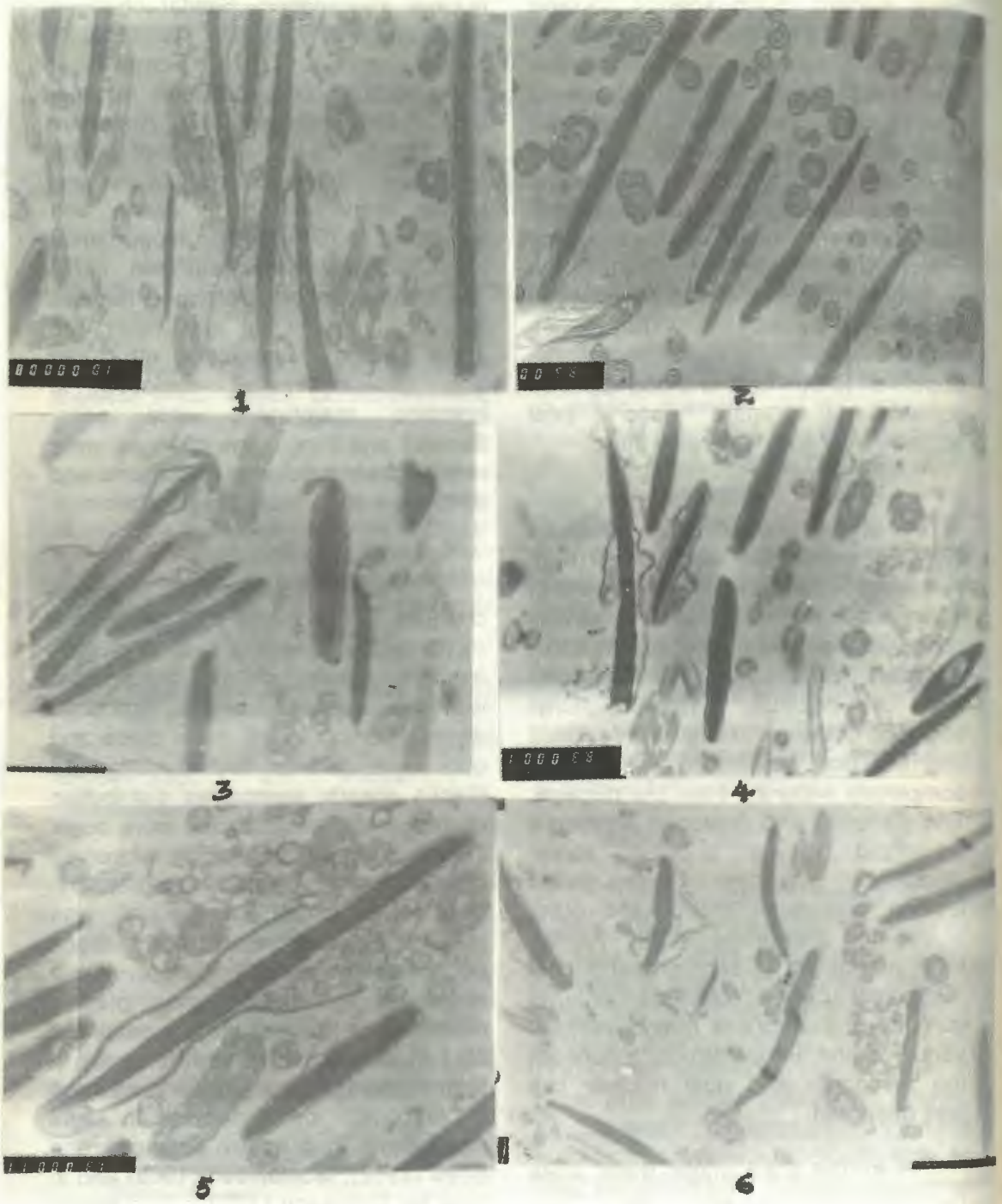
swollen and vacuolated acrosome were evident. Such sperms showed uneven plasma membrane with small vacuoles between the plasma membrane and outer acrosomal membrane. The next significant change noted was the expansion and separation of the plasma membrane over the anterior part of acrosome. The swollen plasma membrane was completely separated from the acrosome and the space in between showed moderately electron dense small vesicles. The acrosome appeared to be intact and normal. The equatorial segment and post acrosomal sheath were also intact.

In the second stage changes involved mainly the acrosome. (Fig). Following complete disruption of plasma membrane the acrosome was observed to be thickened, twisted, distorted, misshapen and detached from the nucleus. The equatorial segment of the acrosome disintegrated and the post acrosomal sheath was completely separate from the nucleus. The distorted and detached acrosome presented vesicle and in some, detached acrosome disintegrated leaving acrosomal matrix and outer acrosomal membrane alone. At the level of equatorial segment and post acrosomal space accumulation of large quantity of membrane bound vesicles vacuoles and fibrous structure beneath the acrosome and post nuclear cap was observed.

In the third stage there were numerous deformed sperm head. (Fig). The nucleus of the spermatozoa presented zones of non-condensed chromotin (Nuclear vacuoles) and nuclear invagination. Similar changes were observed in bovine spermatozoa by Coulter et.al., (1978). Sometime it changed the contour of the nucleus (Bloom and Anderson, 1975, Anderson and Filseth, 1976). Cases of pouch formation in the nucleus of porcine and bovine testicular sperm (Bane and Nicander, 1965) and after experimental cryptorchidism in rabbits (Pleon, 1973) has been reported. Deformed nucleus free of acrosome but associated with small vesicle, filamentous structures and cellular matrix enclosed in common membrane were seen. Second and third stage changes were mostly observed during the second and third week of testicular degeneration.

The observed structural changes in the sperm mid piece were mitochondrial swelling less and non-electron dense matrix, mitchondria without cristae and axonemal disorganization in the main piece and end piece, swollen and disintegrated fibrous sheath, anomalous distribution of axonemes, microtubules were seen. Jones and Martin (1973) observed similar changes in ram seprmatozoa. The sharp folding of the tail was evident by the presence cross sections of main piece at different levels enclosed in the same plasma membrane. Kojima (1977) recorded 60.1 % of hair pin curved deformity in boar spermatozoa under electron microscope, by means of light microscope in bulls by Swanson and Boyd (1962), Gustafsson (1966) and in boar by Einarsson (1971). The sperm mid piece and main piece defects were seen in all the three stages.

Figure: Electron Photomicrographs of sperms after Testicular degeneration in Bucks



1&2:- Stage One : Expanded and separated plasma membrane from intact acrosome
3&4:- Stage Two: Thickened and distended acrosome detached from the nucleus
5 :- Membrane bound vesicles and fibrous structure beneath the thickened and detached acrosome
6: -Stage Three: Deformed sperm nucleus devoid of acrosome and with small vesicle and cellular matrix.

The sequential ultrastructural changes of sperm in three stages has not been reported earlier in bucks. Further accumulation of membrane bound vesicles, vacuoles and fibrousstructure in stage 2 and stage 3 close to the sperm heads could not be explained. If they were the remains of the disintegrated axonemes and fibrous sheath of main piece of the tail then their location very close to sperm head and sometimes enclosed in common membrane remains unexplained

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KEEPING QUALITY OF HOLSTEIN FREISIAN SEMEN FOLLOWING THAWING – INCUBATION AT 37°C, ROOM TEMPERATURE AND 5°C*

G.R ONKARAPPA [1], M. DEVARAJ [2**] and T.G. HONNAPPA [3]

Department of Animal Reproduction,
Gynaecology and Obstetrics
Veterinary College, Hebbal, Bangalore – 24

ABSTRACT

The effect of thawing-incubation temperature on keeping quality of Holstein Friesian semen was assessed to evaluate the fitness of semen for A.I. at field level. Frozen semen was thawed at 37°C for 30 sec, room temperature (26°C) for 1 min and 5°C for 5 min and incubated at the same temperature upto 150 min. The percentage of progressive motility, live count, intact acrosomes and minor sperm abnormalities were evaluated at 0, 30, 60, 90, 120 and 150 min of incubation to assess the semen quality. The mean percentage of progressive motility, live count and intact acrosomes showed significant decline (P≤ 0.05) from 0 min to 150 min of incubation. Based on sperm motility, intact acrosomes and thermal stress test at 37°C, its is opined that semen thawed-incubated at 37°C could be utilized for A.I. upto 90 min.

The artificial insemination has been widely accepted as a means to improve livestock production. The lack of portable liquid nitrogen containers at field level have lead the inseminators to carry thawed semen in the thermos flask either containing warm water (35 – 38°C) or ice water (5°C) to perform A.I. at farmers door steps. This may lead to thermal stress affecting the quality of thawed-incubated spermatozoa. The information on thawing and incubation at 37°C, room temperature and 5°C on keeping quality of spermatozoa is sparse (Pace et.al., 1981; Bhosrekar et.al., 1984; Barth and Bowman, 1988; Chinnaiya end Balakrishnan, 1988 and Bhalde et.al., 1991)

The present study was designed to evaluate the effect of thawing-incubation temperatures (37°C, 26°C and 5°C) on the percentage of progressive motility, live count, intact acrosomes and minor sperm abnormalities. The study also envisaged to indicate the time gap between thawing and A.I in unavoidable delayed utilization.

MATERIALS AND METHODS

Eight ejaculates from each of seven Holstein Friesian bulls totaling to 56 ejaculates were coliected at an interval of 3 to 5 days and evaluated for its quality. The semen was extended in Tris-egg yolk-glycerol (to contain 30 million motile spermatozoa),

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** Author for correspondence.
1. Asst. Director, Central Semen Collection Centre, Dharwad.
2. Assoc. Professor.
3. Asst. Professor.

equilibriated, frozen in French mini straws and stored in liquid nitrogen for a minimum of 10-15 days. The percentage of progressive motility, live count, intact acrosomes and minor sperm abnormalities following thawing-incubation at 37°C for 30 sec (0 min), room temperature (26°C) for 1 min (0 min) and 5°C for 5 min (0 min) and again at intervals of 30, 60, 90, 120 and 150 min were assessed.

The data were analysed by multivariate analysis of variance (ANOVA) and by Fischers protracted least significant difference test (Snedecor and Cochran, 1968).

RESULTS AND DISCUSSION

The thawing temperature and incubation time significantly influenced the quality of spermatozoa. The percentage of progressive motility, live count and intact acrosomes declined progressively ($P \leq 0.05$) from 0 min to 150 min of incubation at all temperatures (Table.)This observation is in agreement with the findings of Chinnaiya and Balakrishnan (1988). The percentage of minor sperm abnormalities significantly increased ($P \leq 0.05$) from 0 min to 150 min of incubation at all temperatures. However, the perusal of Table 1. Indicates that the percentage of progressively motile spermatozoa, live count, intact arosomes and minor sperm abnormalities are within the normal requirement for utilization upto 60, 90 and 120 min of thawing — incubation at 37°C room temperature and 5°C respectively. But the spermatozoan survival with the latter two methods (room temperature and 5°C) was not subjected to thermal stress test at 37°C.

The frozen thawed semen with 7.50 to 10.00 million motile spermatozoa (Bharth, 1989) and 80.00 per cent intact acrosomes (Sokolovskaya et. al., 1981) are recommended for A.I. A few conception reports have also been reported by using thawed — incubated semen at various intervals. Hultnaes (1984) recorded 69.10 per cent 60 days NRR with thawed, incubated semen used between 90-120 min at 35.- 38°C. Chinnaiya and Balakrishnan (1988) recorded NRR of 69.00 per cent with frozen semen inseminated after 30-40 min of thawing incubation at 38°C. Devaraj and Jayakumar (1989) have reported 48.42 per cent conception rate for first insemination in cross-bred cattle under village management by utilizing semen thawed incubated in a thermos flask (35 – 38°C)between 30-45 min. Further, Chinnaiya and Balakrishnan (1988) have recorded a NRR of 59.00 and 65.00 per cent with the frozen semen inseminated after 30-40 min of thawing-incubation at room temperature (25-30°C) and in ice water (4°C) respectively.

The findings of the earlier workers and the percentage of progressive motility and intact acrosomes recorded at 60, 90 and 120 min of thawing-incubation at 37°C room temperature and 5°C respectively in the present study points out the possibility of delayed utilization with normal fertility. However, the spermatozoa in latter two methods room temperature and 5°C of thawing incubation were not subjected stress

test (at 37°C) and hence, the semen thawed-incubated at 37°C appears to be more suitable for delayed utilization upto 90 min. Fertility trials utilizing thawed — incubated semen at different intervals are suggested.

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Table : Effect of thawing –incubation temperature on semen parameters (Mean ± SE) of Hoistein Freisian bulls (n=56)

Parameters & Thawing Incubation Temperature	Incubation intervals (min)					
	0	30	60	90	120	150
Progressive motility						
37°C	49.19±0.78^{xa}	44.19±0.98^{xb}	37.94±0.92^{xc}	33.66±1.33^{xd}	27.58±1.80^{xe}	18.83±1.33^{xf}
Room temp.,	44.73±1.06^{ya}	39.11±0.96^{yb}	34.10±0.10^{yc}	30.35±1.42^{yd}	24.19±1.65^{ye}	18.21±1.50^{yf}
5°C	49.82±0.95^{xa}	49.19±1.03^{za}	42.68±0.98^{zb}	36.78±1.30^{zc}	30.18±1.70^{zd}	23.66±1.30^{ye}
Live Count						
37°C	50.94±0.59^{xa}	49.58±0.63^{xb}	48.08±0.79^{xc}	41.21±0.63^{xd}	37.79±0.63^{xe}	35.44±0.59^{xf}
Room temp.,	49.55±0.38^{ya}	48.12±0.45^{yb}	46.07±0.57^{yc}	43.37±0.61^{yd}	36.35±1.05^{ye}	34.50±0.84^{yf}
5°C	50.11±0.40^{xya}	48.60±0.46^{xyb}	46.53±0.52^{yzc}	43.00±0.51^{yzd}	36.25±0.97^{yze}	32.66±0.95^{yf}
Intact acrosomes						
37°C	77.52±0.97^{xa}	74.37±0.80^{xb}	69.13±0.91^{xc}	62.00±1.15^{xd}	52.49±1.84^{xe}	41.39±1.37^{xf}
Room temp.,	75.25±0.86^{ya}	72.56±0.73^{yb}	65.42±0.89^{yc}	57.55±0.34^{yd}	47.36±0.53^{ye}	39.43±0.45^{yf}
5°C	80.37±0.41^{za}	77.39±0.40^{zb}	72.59±0.38^{zc}	66.35±0.56^{zd}	61.54±0.56^{ze}	45.00±0.39^{zf}
Minor abnormalities						
37°C	11.11±0.07^{xa}	12.03±0.12^{xb}	12.50±0.10^{xc}	13.09±0.06^{xd}	13.00±0.09^{xd}	14.33±0.07^{xe}
Room temp.,	10.51±0.06^{ya}	11.66±0.11^{yb}	12.36±0.07^{yc}	13.18±0.07^{yd}	14.58±0.04^{ye}	15.06±0.14^{yf}
5°C	10.48±0.08^{yza}	10.64±0.08^{za}	13.34±0.08^{yb}	13.71±0.06^{yc}	15.38±0.10^{zd}	16.46±0.09^{ze}

Note: Mean bearning one common superscript either in a row (a,b,c,d,e,f)or a column (x,y,z)in each parameter do not differ significantly (P≤0.05)with each other.

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EFFECT OF CRYOPRESERVATION ON THE VIABILITY AND MEMBRANE INTEGRITY OF CANINE SPERMATOZOA

S.R. MOHANACHANDRAN NAIR, J. KALATHARAN and J. RAJASEKARAN

Madras Veterinary College
Tamilnadu Veterinary and Animal Science University
Chennai

ABSTRACT

Semen samples were collected from adult mongrel dogs by digital manipulation. Two glycerol levels in Tris-egg yolk extender, two equilibration periods and two freezing rates were compared to arrive at an optimum freezing protocol. Post-thaw evaluation was based on motility and hypo-osmotic swelling response to assess the fertilizing ability of spermatozoa. Tris-egg yolk buffer with 9 per cent glycerol level, 4 hour equilibration period and slow freezing rate were found to be optimal for freezing dog semen.

The use of frozen semen technology so far has not been successful in dogs, when compared to other domestic animals. Various factors like the effect of extenders, cryoprotectants and their levels, pH of the buffer, equilibration period and freezing rates are to be investigated to overcome the lowered fertility of frozen semen.

The routine semen analysis based on sperm count, motility and morphology has limitations in predicting the fertilizing ability of spermatozoa. Hypo-osmotic swelling test is reported to be effective in assessing the functional characteristics of spermatozoa and its fertilizing ability (Kumi Diaka, 1993; Correa and Zavos, 1994; Rodriguez-Gill et.al., 1994) The hypo-osmotic swelling response is having high correlation with in vitro fertilization capacity of human spermatozoa (Jayendran et. al., 1984).

A combination of routine semen analysis with hypo-osmotic swelling test will help to develop an optimum freezing technique for cryopreserving the canine spermatozoa. The objective of the present study is to compare effect of two glycerol levels, two equilibration periods and two freezing rates on the post thaw motility and hypo-osmotic swelling response of canine spermatozoa extended in Tris-egg yolk diluent.

MATERIALS AND METHODS

Five adult healthy male mongrel dogs were selected for this study. All the dogs were maintained on a uniform balanced diet. The sperm-rich fractions of the ejaculate were collected by digital manipulation (Allen, 1991), without the presence of a teaser bitch. The volume, colour and consistency was evaluated immediately after collection. The percentage of progressively motile spermatozoa were estimated under phase contrast microscope. The hypo-osmotic swelling (HOS) bio assay was

performed by first mixing I ml of 150 milli osmol fructose in a test tube with 0.1.ml of ejaculate and incubating the mixture for 30 minutes at 37°C(England and Plummer, 1993). After incubation smears were prepared on glass slides, dried and stained with buffered Rose Bengal stain. Percentage of hypo-osmotically swelled spermatozoa which exhibit curling of tail were estimated. Straight or slightly curved tails were taken as not swollen.

Technique of Freezing : Tris citric acid fructose buffer with 20% egg-yolk (Anderson, 1975) was used to extend the semen samples. Buffer pH was adjusted to 7.0. Two glycerol levels, 9 per cent and 6 per cent were compared. In order to achieve minimum sperm density of 100 millions/ml (Christiansen, 1984) dilution rate 1:2 was used. Extended samples were filled in 0.25 ml plastic straws and transferred to cold handling chamber at 5°C. Two equilibration periods 2 hours and 4 hours were compared in the study. After equilibration period slow freezing was achieved by keeping straws 10 cm above liquid nitrogen level for 20 minutes and fast freezing by keeping straws 5 cm above liquid nitrogen level for 5 minutes. Frozen straws were stored in liquid nitrogen. Thawing was done in water bath at 40°C for one minute. Post-thaw motility and hypo-osmotic swelling response were evaluated. The data on sperm motility and hypo-osmotic swelling response for 3 factors and their interactions were studied by subjecting the data into 3 factor completely randomized design as suggested by Kampthorne (1967).

RESULTS AND DISCUSSION

General seminal characteristics of fresh dog semen is shown in Tabe I. The effect of cryopreservation on the functional characteristics of spermatozoa are expressed as mean percentage (± SE) in table II. Post thaw motility and hypo-osmotic swelling response showed significant difference between fresh and frozen thawed semen ($P \leq 0.01$). Post –thaw motility (34.74 ± 4.32%) and HOS response (50.69 ± 2.07%) were significantly higher ($P \leq 0.01$) for extender having 9% glycerol compared to 6 per cent glycerol. Longer equilibration period of 4 hours showed better post-thaw motility (32.4 ± 4.42%) and HOS response (48.67 ± 2.07%) when compared to 2 hours equilibration period.

Slow freezing rate resulted in significantly higher post-thaw motility (46.5 ± 1.9%) and HOST response (52.88 ± 1.7%) compared to fast freezing rate. The study on the effect of glycerol levels on the post-thaw viability of spermatozoa revealed that a higher level of 9% glycerol level was suitable for freezing and in accordance with findings of Foote. (1964), who achieved optimum post-thaw motility with 11 per cent glycerol in Tris-buffered Egg Yolk glucose extender and Smith (1984) with 9 per cent glycerol in Egg Yolk – Pipes extender for cryopreservation of dog semen. However, contradictory results were obtained by Olar et.al., (1989) who found that post-thaw motility was highest with 2-4% glycerol level in Egg yolk-Tris based diluent. Higher percentage of hypo-osmotically swollen

spermatozoa in the present study indicate that higher glycerol level preserve the fertilizing capacity of spermatozoa during freezing.

Longer equilibration period was found to be having significantly higher ($p \leq 0.01$) post-thaw motility and hypo-osmotic swelling response. This allowed time for membrane changes in ionic fluxes, making the membranes of the spermatozoa resistant to cooling (Watson,1975).

A slow freezing rate was found superior compared to fast freezing rate. Fast freezing rate resulted in very low post thaw motility in studies by Olar et.al., (1969) and Dobrinski et. al., (1993). Significantly lower ($p \leq 0.01$) HOS response of spermatozoa in fast freezing rate in the present study concurs with these observations.

Developing freezing protocols based on post-thaw motility alone is not the best technique since it did not evaluate the sperm fertilizing capacity after freezing and thawing. Hypo-osmotic swelling test is a simple and economical bio-assay so accurately predict the fertilizing ability of spermatozoa (Jeyendran et.al., 1984; Correa and Zavos, 1994 and Rodriguez – Gill et.al., 1994) Significant reduction in motility and HOS response will contribute to poorer fertility of frozen semen compared to fresh and chilled semen. But satisfactory fertility can be achieved by intra-uterine deposition of frozen semen and accurate determination of timing of ovulation.

Table.1

Seminal characteristic in Dogs

Characteristics	Mean Value (± SE)
Volume (ml)	
Fraction - I	0.82 ± 0.17
Fraction - II	1.47 ± 0.11
Fraction - III	5.92 ± 0.86
Colour & Consistency	
Fraction - I	clear watery
Fraction - II	creamy, medium to thick
Fraction - III	clear watery
Second Fraction :	
Motility %	87.33 ± 0.91
Concentration 10^6 per ml	313 ± 30.74
Live sperms %	84.06 ± 1.27
Abnormal sperms %	7.61 ± 0.18
Acrosomal damage %	2.16 ± 0.23
HOS positive %	91.47 ± 2.04

Table .II

Effect of Cryopreservation on the functional Characteristics Of canine Spermatozoa (Mean Percentage ± SE)

Factors	Post-thaw Motility	Hypo-osmotic Swelling Positive –spermatozoa
EFFECT OF CLYCEROL LEVELS		
6 percent glycerol	25.99 [a] ± 3.28	43.36 [a] ± 1,21
9 per cent glycerol	34.74 [b] ± 4.32	50.69 [b] ± 2.07
EFFECT OF EQUILIBRATION PERIODS		
2 hour equilibration	28.33 [a] ± 3.39	45.39 [a] ± 1.61
4 hour equilibration	32.40 [b] ± 4.42	48.67 [b] ± 2.07
EFFECT OF FREEZING RATES		
Slow freezing	46.5 [a] ± 1.9	52.88 [b] ± 1.7
Fast freezing	14.23 [b] ± 0.67	41.18 [b] ± 0.83

a,b, Means with different super scripts in the same column differ ($p \leq 0.05$)

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EFFECT OF TESTICULAR DEGENERATION ON THE BIOCHEMICAL AND ENZYME CONSTITUENTS OF BUCK SEMEN*

D. ANTOINE** and S.R. PATTABIRAMAN

Department of Obstetrics and Gynaecology
Madras Veterinary College
Chennai 600 007.

ABSTRACT

Healthy Tellicherry bucks aged 3 years were subjected to scrotal insulation for 48 hrs. 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. The GOT and GPT levels showed declining trend from 2nd week but however it remained at higher level upto 12th week than the control animals and then it reached near normal level by 13th week. The total protein content in the seminal plasma was markedly reduced during first week post-scrotal insulation. The albumin level was significantly high during the first three weeks post treatment.

Testicular degeneration is one of the most common cause of reduced fertility or sterility in male domestic animals. Scrotal insulation technique was adoped for experimental induction of testicular degeneration in bulls (Lagerlof, 1934) and in rams (Rao and Rao, 1977). The present investigation was taken up to study the effect of scrotal insulation induced testicular degeneration on the bio-chemical and enzyme constituents of semen.

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** Asso. Professor Department. of obstetrics and Gynaecology Rajiv Gandhi college of veterinary and Animal Science, Pondicherry.

MATERIALS AND METHODS

Six Tellicherry bucks aged 3 years were subjected to scrotal insulation for 48 hrs. The scrotal bag was made of double walled lint material with non-absorbable cotton and glass wool. 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. The concentration of glutamic oxaloacetic trasaminase (GOT) glutamic pyruvic transminase (GPT) enzymes and total protein and albumin in the seminal plasma were estimated for 7 post treatment weeks by colorimetric method as per the Reitman and Frankel (1957) and modified Biuret and Dumas method (Dumas et.al., 1981)

RESULTS AND DISCUSSION

The level of GOT and GPT in Pre-treated control bucks was 117.02 ± 1.81 and 31.99 ± 1.81 units/ml. During first week scrotal insulation GOT and GPT level increased significantly to 393.99 ± 69.30 units/ml and 125.06 ± 25.5 units/ml respectively (Table.1). Though the level of Transaminase showed a gradual decline from 2nd week to 12th week for GOT and 2nd week to 10th week for GPT, they

remained significantly higher than pre-treatment control. The decline in the level of transaminase could be due to decrease in the spermatozoal concentration. Jagir Singh et.al., (1991) concluded that the higher level of GOT and GPT in seminal plasma could be due to the presence of varying proportion of ageing and decaying spermatozoa in the ejaculate, difference in Testosterone level (Mann and Lutwak–Mann ,1981) and variation in secretion from accessory sexual glands (Pace and Graham, 1970). In this study it is evident that heat stress caused by scrotal insulation has resulted in structural damage of sperm so that the enzyme is released in significant quantity in seminal plasma.

The total protein in the seminal plasma in pre-treatment control bucks was 3.86 ± 0.20/100ml. Dunder et.al., (1983) recorded 2.99 ± 0.28 g of total proteine in seminal plasma of Angora bucks. During 1st week of post-scrotal insulation the level significantly ($p<0.01$) reduced to 1.57 ± 0.26 gm/100 ml. The variation in the level of total protein from 2nd to 7th week was not significant.

The albumin level in the seminal plasma increased highly significantly upto 3rd week post treatment period. Thereafter the level declined and reached normal level by 6th week. The globulin level significantly reduced during first two weeks post treatment. Thereafter a gradual non-significant increase was noticed. Albumin and globulin ratio clearly shows highly significant increase in albumin concentration upto 3 week post treatment (Table.2).

Normaly seminal plasma contained more of globulin than albumin. High level of albumin in seminal plasma was associated with infertility (Mann and Lutwak Mann. 1981). Hence the high level of albumin from 1st to 3rd week post scrotal insulation renders the semen sample unfit for use.

Table-1.

Effect of Testicular degeneration of GOT & GPT level in seminal plasma.

Treatment/Period	GOT units/ml ± SE	GPT units/ml ± SE
Pre-treatment control	117.02 ± 1.81	31.99 ± 1.81
Post-treatment period (weeks)		
1	393.99 ± 69.3 **	125.06 ± 25.50**
2	283.97 ± 32.52 **	115.29 ± 8.63**
3	273.49 ± 58.69 **	100.77 ± 5.29 **
4	151.71 ± 27.65 **	86.59 ± 10.61 **
5	220.71 ± 53.65 **	76.47 ± 11.44 **
6	221.49 ± 85.81 **	95.49 ± 10.27 **
7	160.46 ± 22.40 **	75.70 ± 2.20 **
8	197.73 ± 11.40 **	75.21 ± 1.11 **
9	190.43 ± 3.16 **	73.00 ± 1.88 **
10	152.34 ± 9.31 **	59.68 ± 4.34 **
11	133.84 ± 2.22 **	40.54 ± 6.33 NS
12	130.14 ± 3.27 *	34.97 ± 4.49 NS
13	123.17 ± 5.04 NS	29.99 ± 1.83 NS

Table.2

Effect of Testicular degeneration on certain biochemical constituents of Seminal plasma

Treatment / Period	Total Protein gm %	Albumin gm	Globulin gm	A/G ratio
Pre-treatment control	3.86 ± 0.20	1.04 ± 0.08	2.82 ± 0.16	0.36 ± 0.04
Post-treatment Period (weeks)				
1	1.57 ± 0.26 **	0.92 ± 0.18 **	0.65 ± 0.11 **	1.45 ± 0.26 **
2	3.58 ± 0.57 NS	2.92 ± 0.15 **	0.66 ± 0.50 **	4.42 ± 0.33 **
3	4.70 ± 1.07 NS	2.76 ± 0.40 **	1.94 ± 0.73 NS	1.42 ± 0.13 **
4	2.61 ± 0.53 NS	0.76 ± 0.23 **	1.85 ± 0.45 NS	0.41 ± 0.15 NS
5	2.66 ± 0.80 NS	0.58 ± 0.08 **	2.08 ± 0.75 NS	0.27 ± 0.13 NS
6	2.86 ±1.25 NS	0.82 ± 0.55 NS	2.04 ± 0.79 NS	0.40 ± 0.04 NS
7	3.94 ±0.63 NS	1.02 ± 0.22 NS	2.92 ± 0.45 NS	0.34 ± 0.04 NS

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EFFECTS OF VARIOUS LEVELS OF GLYCEROL AND EGG YOLK IN FREEZING OF ONGOLE BULL SEMEN*

G. VEERAIAH [1], K. VENUGOPAL NAIDU [2], and K. BABU RAO [3]

Department of Animal Reproduction and Gynaecology
College of Veterinary Science, Tirupati – 517 502
Andhra Pradesh.

ABSTRACT

The highest percentage of Prefreeze motility (71.80), Post thaw motility (53.61), live sperms (56.06) and acrosomal integrity (88.14) was found in Tris diluent containing 6 per cent glycerol with 10 per cent egg yolk than in diluent with other combinations of glycerol and egg yolk. The per cent of prefreeze motility (69.94), post thaw motility (36.11), live sperms (37.87) and acrosomal integrity (69.97) were found to be significantly low in this diluent containing 8 per cent glycerol with 20 per cent egg yolk. Significant difference was noticed between various combinations of glycerol with egg yolk in prefreeze motility, post thaw motility, live sperms and acrosomal integrity. The overall means of the above parameters were significantly high with 6 per cent glycerol than 4 and 8 per cent glycerol. The Tris diluent containing 6 per cent glycerol with 10 per cent egg yolk was best for freezing of Ongole bull semen.

Chaves (1979) demonstrated the adverse effect of freezing and thawing on acrosomal integrity. Kumar (1989) reported the effect of yolk levels on post thaw live sperms and acrosomal integrity. Most of the authors have confined their investigation to post thaw motility only for evaluation of freezability. Hence, the present study was undertaken to observe the effect of various levels of glycerol and egg yolk on freezing of Ongole bull semen and to standardise the optimum levels of glycerol and egg yolk in the diluent.

MATERIALAS AND METHODS

The present study was undertaken at Cattle Project, Livestock Research Station, Lam, Guntur. A total of 36 ejaculates from six fertile Ongole bulls aged between 4 to 6 years were used for this study. A factorial design of three levels of glycerol (4.6 and 8 per cent) and three levels of egg yolk (10.15 and 20 per cent) in Tris diluent was used. After initial evaluation, the semen was extended at 37°C with freshly prepared Tris diluents having different levels of glycerol and egg yolk and cooled to 5°C over about an hour. Then the semen was loaded in 0.5 ml straws and equilibrated for 5 hours before freezing in liquid nitrogen. The straws were thawed at 37°C for 30 seconds after 24 hours of freezing. The prefreeze motility, post thaw

1. Vety.Asst.Surgeon, Veterinary Despensary, Chodavaram, West Godavari Dist. (A.P.)
2. Associate Professor
3. Research Officer, Cattle Project, Lam, Guntur.

* Part of M.V.Sc. thesis submitted by first author to the Andhra Pradesh Agricultural University , Hyderabad.

motility, live sperms and acrosomal integrity was assessed as per the standard procedures. The data was analysed statistically as per Snedecor and Cochran (1969).

RESULTS AND DISCUSSION

The mean percentage of prefreeze motility at 4 per cent and 6 per cent glycerol in combination with 10, 15 and 20 per cent of egg yolk, did not differ significantly (Table.1). However, the overall percentage of prefreeze motility was declined significantly in dilutor containing 8 per cent of glycerol with 20 per cent egg yolk. The decline in prefreeze motility with increasing percentage of glycerol (Table.2) recorded in this study might be due to adverse effect of glycerol on sperms as reported by Jayaraman et.al., (1979). No significant difference in prefreeze motility was noticed in Tris diluent containing various levels of (10, 15 and 20 per cent)of egg yolk (Table.2). The absence of depressing effect on prefreeze motility observed in the case of varying proportions of egg yolk may be due to the combined effect of cholesterol and lecithin present in yolk in protecting the sperms from cold shock as reported by Lanz et.al., (1965)

The percentage of post thaw motility observed in this investigation were found to be similar with the observations of Sagdeo et.al., (1982) and Singh et.al., (1993). The maximum post thaw motility of 53.61 per cent was observed when a combination of 6 per cent glycerol with 10 per cent egg yolk was used in Tris diluent (Table 1) in the present study. The second highest post thaw motility of 47.78 and 46.39 was recorded in case of 6 per cent glycerol with 15 per cent yolk and 4 per cent glycerol with 10 per cent yolk respectively. The lowest percentage of post thaw motility (36.11) was estimated in Trist diluent containing 8 per cent glycerol with 20 per cent yolk. The post thaw motility significantly differed between various levels of glycerol and egg yolk (Table.2) The higher post thaw motility was observed in Tris diluent with 6 per cent glycerol (47.82%) than 4 per cent (42.32%) and 8 per cent (39.86 %) glycerol. Becker et.al., (1977), Gilbert and Almquist (1978) and Kumar et.al., (1994) substantiated that less proportion of glycerol (about 6%) was beneficial for higher percentage of post thaw motility. Maximum post thaw motility was observed with 10 per cent egg yolk (47.78 %) than with 15 per cent (43.06%) and 20 per cent (38.17%) egg yolk. The decrease in post thaw motility 20 per cent egg yolk level observed during this investigation was found to be in agreement with Zarazua et.al., (1977) and Kumar et.al., (1994).The depression in motility at high concentration of 20 per cent egg yolk might be due to aggregation of lecithin molecules, rendering the lecithin unavailable for binding with plasma membrane of sperms as pointed out by the Lanz et.al., (1965). The maximum percentage of post thaw live sperms of 56.06 was recorded with 6 per cent glycerol and 10 per cent egg yolk levels in this study (Table.1). The lowest percentage of post thaw live sperms of 37.87 was observed when Tris dilutor containing 8 per cent glycerol and 20 per cent egg yolk was used. The trend in post thaw

percentage of live sperms for various combinations of glycerol and egg yolk was found to be similar with respect of post thaw motility discussed earlier. The percentage of post thaw live sperms observed in this investigation was found to be slightly higher than the percentage of post thaw motility. The above mentioned observation could be explained in view of all the live sperms may not exhibit progressive motility. Similar difference in between the percentage of post thaw motility and live sperms was reported by Misra et.al., (1988).

The percentage of the post thaw acrosomal integrity observed during this investigation was found to be in agreement with the results of Becker et.al., (1977).Gilbert and Almquist (1978) and Kumar (1989). The percentage of post thaw acrosomal integrity was found to be highest (88.14%) in Tris diluent containing 6 per cent glycerol with 10 per cent egg yolk and lowest (69.97%) in Tris diluent containing 8 per cent glycerol with 20 per cent yolk (Table.1). The mean percentage of acrosomal integrity was observed to be best in Tris diluent containing 6 per cent glycerol (85.90%) than 8 per cent (72.48%) and 4 per cent (80.28%) glycerol (Table.2) Becker et.al., (1977) also recorded best acrosomal integrity in Tris dilutor with 7 per cent glycerol than with 9 and 11 per cent glycerol.. A significant drop in acrosomal integrity was also observed with ascending levels of egg yolk in this investigation. Similarly Kumar (1989) observed significant drop in acrosomal integrity as yolk level was increased from 5 to 20 per cent in bufallo bull semen frozen in Tris diluent.

Table.1 Effect of different levels of Glycerol and Egg yolk on Freezing of Ongole bull semen

Parameters	4 per cent Glycerol			6 per cent Glycerol			8 per cent Glycerol		
	10 per cent yolk	15 per cent yolk	20 per cent yolk	10 per cent yolk	15 per cent yolk	20 per cent yolk	10 per cent yolk	15 per cent yolk	20 per cent yolk
Pre freeze motility	71.39^{a} ± 0.50	70.97^{ab} ± 0.51	70.83^{ab} ± 0.60	71.80^{a} ± 0.71	71.39^{a} ± 0.92	70.69^{ab} ± 0.96	69.58^{ab} ± 0.86	68.47^{b} ± 1.09	66.94^{c} ± 1.25
Post thaw motility	46.39^{a} ± 0.58	41.25^{avc} ± 0.78	39.31^{c} ± 0.82	53.61^{a} ± 0.93	47.78^{b} ± 0.61	42.08^{cd} ± 0.89	43.34^{c} ± 0.54	40.14^{dc} ± 0.54	36.11^{f} ± 0.54
Post thaw live sperms	49.78^{b} ± 0.66	44.53^{c} ± 0.96	42.36^{cd} ± 0.91	56.06^{a} ± 0.83	48.75^{v} ± 0.67	44.69^{c} ± 1.00	43.55^{cd} ± 0.73	41.06^{d} ± 0.73	37.87^{c} ± 0.76
Post thaw acrosomal integriy	83.31^{d} ± 0.35	80.59^{c} ± 0.27	77.95^{f} ± 0.18	88.14^{a} ± 0.24	86.31^{b} ± 0.24	83.35^{c} ± 0.47	74.81^{g} ± 0.19	72.67^{b} ± 0.30	69.97^{f} ± 0.34

Table 2 Overall Effect of Different Levels of Glycerol and Egg yolk on freezing of Ongole bull semen.

Parameters	Percentage of Glycerol			Percentage of Egg yolk		
	4	6	8	10	15	20
Pre freeze motility	71.07[a]	71.30[a]	68.33[v]	70.93[a]	70.28[a]	69.49[a]
Post thaw motility	42.32[b]	47.82[a]	39.86[c]	47.78[a]	43.06[b]	39.17[c]
Post thaw live sperms	45.55[b]	49.83[a]	40.83[c]	39.80[a]	44.78[b]	41.64[c]
Post thaw acrosomal integrity	80.28[b]	85.90[a]	72.48[c]	81.75[a]	79.86[b]	77.08[c]

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SEXUAL BEHAVIOUR, SEMINAL CHARACTERISTICS AND FERTILITY IN STALLION

CAPT. VINAYA DIXIT and R.K. PANDIT

Department of Obstetrics and Gynaecology
College of Veterinary Science and Animal Husbandry
Jabalpur, Madhya Pradesh 482 001.

ABSTRACT

Six stallions were studied for body weight (498.33 ± 18.07 kg), scrotal circumference (35.3 ± 1.28 cm), reaction time (4.45 ± 0.18 min), mounts per ejaculate (1.58 ± 0.08), copulation time (27.62 ± 0.49 sec), semen value (44.75 ± 1.88 ml), semen pH (7.27 ± 0.01) sperm motility (72.61 ± 0.53) sperm concentration (295.31 ± 14.74 x 10^6/ml), total sperm per ejaculate (11.98 ± 0.43 x 10^9), live sperms (74.27 ± 0.57 %) normal sperms (81.57 ± 0.57 %) and length of sperms (59.44 ± 0.04 µm). Totally, 144 mares were bred artificially to the stallions and obtained 43.06 % first service conception. The coefficient of correlation between sexual behaviour and different seminal characteristics was studied.

In equines the natural breeding season is during spring and summer. Furthermore, stallion expells large number of sperms in each ejaculate and thus depletes epididymal reserves quickly. Therefore, artificial breeding is recommended specially during the breeding season so as to cover maximum number of mares and prevent over use of stallions by natural breeding. For artificial breeding it is necessary to know the sexual behaviour, seminal characteristics and fertility pattern in equines. Hence, these traits were studied in the Army stallions and mares of Saharanpur farm.

MATERIALS AND METHODS

Six breeding stallions (Equs caballus) and 144 mares belonging to the Remount Training school, Saharanpur, were included in the study. The age of stallions ranged from 6 to 9 years. The body weight and scrotal circumference of the stallions was recorded. The managemental practices were as per the routine farm standard. Ten ejaculates were collected at 2 days interval by artificial vagina. (Pickertt and Back, 1973) with the recording of sexual behaviour, semen volume and pH. From the gel free ejaculate, 2 ml aliquot was kept for evaluation and rest was diluted. (Singhvi, 1990). Thereafter, the sperm motility, concentration, total numbar per ejanculate, live percent, morphology and biometery were recorded (Pickett and Rock, 1973).

The oestrus detection was done by using a teaser. The cervix was opened by one finger, and a pipette was guided into the uterus and 10 to 20 ml diluted semen with atleast 500 million progressively motile spermatozoa was deposited. The pregnancy was diagnosed gynaeco clinically 40 days post insemination.

The fertility was reckoned by percent mares pregnant of the total inseminated.

RESULTS AND DISCUSSION

The mean body weight (498.33 ± 18.07 kg), scrotal circumference (35.3 ± 1.28 cm) reaction time (4.45 ± 0.18 min) mounts per ejaculate (1.58 ± 0.08), copulation time (27.62 ± 0.49 sec), semen volume (44.75 ± 1.88 ml), semen colour (Milky white), pH (7.27 ± 0.01), sperm motility (72.61 ± 0.53), concentration (295.31 ± 14.74 x 10^6/ml), total sperms per ejaculate (11.98 ± 0.43 x 10^9), live sperms (74.27 ± 0.57 %) and normal sperms (81.57 ± 0.57%) were within the normal range as reported from abroad (Roberts, 1986 and Hefez, 1993). Variation has been reported in the body weight and scrotal circumference depending on age of the stallion. The testicular size and weight increases and decreases during breeding and non breeding seasons, respectively (Thompson et.al., 1979). The sexual behaviour and seminal characteristics in equines are affected by rough handling, over use, excessive teasing, age, poor management, isolation, etc., (Pickett and Voss, 1975).

Amongst the sperm abnormalities (18.43 ± 1.38%), the maximum were tail (10.12 ± 1.08%) followed by proximal protoplasmic droplets (2.01 ± 1.17%) loose heads (1.89 ± 0.77 %) abnormal mid pieces (1.14 ± 0.09 %), distal protoplasmic droplets (1.51 ± 0.13 %) and abnormal sperm head (1.76 ± 0.39 %). Reports by other investigators are comparable to the present findings (Dowsett et.al., 1984). The length of sperm head (6.82 ± 0.01μ m) and tail (52.62 ± 0.03 μm) varied significantly (P.∠ 0.01) amongst the stallions which is in agreement with Nishikawa et.al., (1951).

The coefficient of correlation of body weight with scrotal circumference, semen volume and reaction time was positive, whereas it was significantly negative (P .∠ 0.01) between semen volume and sperm concentration (0.665). The sperm concentration was significantly correlated (P..∠ 0.05) to live sperms (0.253). Whereas, total sperms per ejaculate were significantly correlated (P.∠ 0.01) to semen volume (0.336). The first service conception rate was 43.06%

Dott (1975) reported a positive correlation between normal sperms and fertility, Dowsett and Pattie (1982) observed that in stallions, increase in semen volume, sperm concentration, total sperms per ejaculate and total number of live spermatozoa increases the conception rate.

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SPERMIOGRAM AND BIOCHEMICAL STUDIES IN MURRAH BUFFALO BULLS*

NARAYAN PRATAP* V.N. VISHWANATHA REDDY, P.A. SHARMA, T.G. HONNAPPA, M. DEVARAJ, A. KRISHNASWAMY and V.K. ARORA

Department of Gynaecology and Obstetrics,
Veterinary College U.A.S.
Hebbal Bangalore – 560 024.

The fertility of a bull depends solely on the quantitative and qualitative characteristics of the semen produced. Thus, the spermiogram of a bull is of prime importance and helps in assessing the fertility of that bull. The present investigation was carried out at the Central Frozen Semen production and Training Institute. Hessaraghatta, Bangalore to enable the preparation of the spermiogram and assess membrane integrity of spermatozoa in Murrah bulls.

MATERIALS AND METHODS

Semen from six adult Murrah buffalo bulls aged 40 to 62 months was collected with an artificial vagina. Six ejaculates from each bull were collected and subjected for macroscopic and microscopic examination. Semen volume was noted from the graduated semen collection tube. The mass activity and initial motility were graded as per Tomar et.al., (1966). Sperm concentration was estimated by use of a Bovine photometer, IMV. France. The percentage of live count was estimated by means of differential staining technique (Blom, 1950). The per cent intact acrosomes was assessed as per the methods described by Watson (1975). Major and minor sperm abnormalities were assessed by employing the classification of Blom (1972). The concentration of transaminases namely Aspartate amino transaminase (AST) and Alanine transaminase was estimated in seminal plasma obtained after centrifugation by KIT method (Rashmi diagnostics, Bangalore) by employing an auto-analyser BT 224 (Biotechnica instruments, Italy) as per the preocedure laid down in their hand out. The integrity of the sperm plasma membrane was assessed by using the HOST, where in 1.0 ml of hypoosmotic medium consisting of equal quantities of fructose and sodium citrate with an ionic strength of 0.15 (150 milli osmoles) was mixed with 0.1 ml of neat semen as per the procedure described by Jeyendran et.al., (1984). The HOST medium was prepared by adding 7.35 grams of sodium citrate and 13.51 grams of fructose into 1000 ml of distiled water. All estimations were done in prefreeze samples. The data was statistically analysed using standard statistical methods (Snedecor and Cochran, 1968).

* Part. Of M.Sc. thesis submitted to UAS.Bangalore by the first author, Present address: Technical Officer, CFSP and TI Hessaraghatta, Bangalore – 580 088. India.

RESULTS AND DISCUSSION

The mean values of different seminal attributes studied pre-freeze are given in Table. The mean volume of semen from Murrah buffalo bulls under study, concurs with Yadav and Sharma (1996). The mean mass activity of experimental bulls was similar to that reported by Chauhan et.al., (1991).

The mean sperm concentration in ejaculates was similar to the findings of Murugavel (1997). The mean progressive initial motility and mean live count were similar to that of Goyal et.al., (1996) for Murrah breed. The mean percentage of intact acrosomes are similar to those reported by Bhosrekar et.al., (1993). The mean percentage of major and minor sperm abnormalities were found to be well within the acceptable range. Although there are no reports on the study of transaminases by employment of an auto analyser in Murrah bulls, the enzyme activity of the present study are supported by the work of Bhosrekar (1994).

The percentage of spermatozoa that reached to the HOST was found to be a good indicator of sprmatozoa with an intact plasma membrane and only spermatozoa with an intact plasma membrane exhibited swelling and coiling of tail. This indicates that buffalo bull spermatozoa did react to HOST, similar to that of canine, cattle and could prove to be a useful technique to study the sperm membrane integrity and be employed as an invitro fertility test. As there are no previous reports on the employment of HOST in buffalo bull spermatozoa the values could not be compared, however, similar findings have been recorded by Correa and Zavos (1994) in cattle semen.

Table : Various seminal atributes of Murrah buffalo bulls

Seminal atributes	Mean ± S.E.	Range
Volume (ml)	4.34 ± 0.48	1.50 to 9.50
Mass activity (0 to 5 scale)	2.77 ± 0.08	1.50 to 3.50
Concentration (millions/ml)	1053.28 ± 91.93	350.00 to 2152.00
Initial Motility (%)	68.32 ± 0.77	60.00 to 80.00
Intact acrosomes (%)	90.60 ± 0.53	84.00 to 96.00
Live count (%)	73.47 ± 0.82	54.00 to 87.00
Morphological abnormalities		
(a) Major (%)	2.79 ± 0.29	1.93 to 3.71
(b) Minor (%)	6.92 ± 0.17	6.20 to 7.34
Transaminases		
(Kinetic units/60 X 10^8 spermatozoa)		
(a) AST (GOT)	18.19 ± 1.41	13.11 to 30.05
(b) ALT (GPT)	1.76 ± 0.12	0.35 to 5.30
Hypo osmotic swelling test (%)	70.21 ± 3.05	47.00 to 83.00

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ESTIMATION OF TRANSAMINASES (AST AND ALT) IN CRYOPRESERVED MURRAH BUFFALO SEMEN *

NARAYAN PRATAP, V.N. VISHWANATH REDDY, P.A. SHARMA, T.G. HONNAPPA, M. DEVARAJ, A. KRISHNASWAMY and V.K ARORA

Department of Gynaecology and Obsietrics, Veterinary College
UAS Bangalore – 560 024.

When the sperm cell is injured its membranes are either inactivated or destroyed, leading to loss of cellular materials. Most of the transaminases were initially found in the sperm cell and the amount of enzyme left in the cell after freezing was important to fertility, concentration of transminases increased in the extracellular medium when semen was plunged into liquid nitrogen. (Graham and Pace, 1967 ; Pace and Graham, 1970). The present study was conducted to assess the concentration to transaminases AST and ALT in Murrah buffalo semen following cryopreservation by employing either conventional freezing or two rates of programmable freezing (Moderate or slow rate) by use of diagnostic kits and the levels studied by an Auto analyser. BT.224 and then compared to their prefreeze values.

MATERIALS AND METHODS

Murrah buffalo bulls (six) aged between 3 and 5 years, maintained under identical conditions were used in a weekly semen collection program by A.V. technique for a period of six weeks

Immediately after collection semen was evaluated for routine macroscopic and microscopic examination for study of the spermiogram. In addition, the concentration of transaminases (AST and ALT) and the membrane integrity test (Hypo osmotic swelling test) were studied.

For assay of concentration of transaminases (AST and ALT), semen samples on dilution and after freezing and thawing following conventional freezing and two rates of programmable freezing (Moderate freezing rate: At -5^{o}c/mt from $+4^{o}$c to -12^{o}c followed by at -60^{o}c/mt from -12^{o}c to -140^{o}c and then plunged into liquid nitrogen (-196^{o}c). Slow freezing rate : At -4^{o}c/mt from $+4^{o}$c to -30^{o}c followed by at -60^{o}c/mt from -30^{o}c to -140^{o}c and then plunged into liquid nitrogen) were centrifuged at 4000 RPM for a period of 8 to 10 minutes to collect the extracelluar fluid. The activity of AST and ALT were determined by use of an Auto analyser (Photometer BT 224, Biotechnica Instruments, Italy) and ready to use diagnostic kits (M/s Rashmi diagnostics, Bangalore) according to the procedure detailed in their handout. The assay was based on the actual kinetic study by measuring the

* Part of M.V.Sc.thesis submitted to UAS, Bangalore, by the first author; Present address : Technical Officer CFSP and TI, Hessaraghatta, Bangalore – 560 088, India.

molecules of enzyme at the peak of activity and was expressed in kinetic units (KU) per 60 X 10^6 spermatozoa. The data generated was tabulated based on rate of freezing employed. The mean and standard error were compared as per the method described by Snedecor and Cochran (1968) and subjected to multivariate analysis of variance.

RESULTS AND DISCUSSION

The overall mean enzyme activity of transaminases AST and ALT prefreezing in Murrah buffalo bulls semen was found to be 18.18 ± 1.41 and 1.76 ± 0.11 KU per 60 X 10^6 spermatozoa respectively. The present study also revealed the post thaw value for AST and ALT to be 42.12 ± 2.91 and 3.15 ± 0.33; 38.33 ± 4.03 and 3.13 ± 0.38; 39.91 ± 4.12 and 3.27 ± 0.30 Kinetic Units per 60 X 10^6 spermatozoa following conventional freezing, moderate rate and slow rate of programmable freezing respectively. While, there was a significant increase ($P \leq 0.005$) in the post thaw concentration of transaminases (AST and ALT) as compared to their respective prefreeze values, however no significant difference was observed in their concentration post thaw in between the method of freezing or the rate of freezing employed.

The enzyme activity in the present study is supported by the works of Bhosrekar (1975) and Chinnaiya et.al., (1979). Findings in the present study clearly indicate that there is cryoinjury subsequent to freezing and thawing of sperm cell which resulted in an increase in the concentration of transminases extracellularly.

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NUCLEIC ACID CONCENTRATIONS IN SPERM CELL PACK OF PATANWADI RAMS AND TEHIR CROSSES

SURESH KUMAR, V.P. VADODARIYA and F.S. KAVANI

Department of Obstetrics and Gyanecology
College of Veterinary Science and Animal Husbandry
Gujarat Agricultural University Sardar Krushinagar.

The importance of DNA associated with the genetic material and RNA involved in protein synthesis, is well known. The present work was made to understand the norms of DNA and RNA contents in indegenous Patanwadi rams and their crosses with various levels of exotic inheritance.

MATERIALS AND METHODS

Present study was undertaken on eighteen healthy breeding rams divided into three groups viz., Group.I.Indigenous patanwadi Rams (6). Group.II Patanwadi half-breds (½ P x ½ M or ½ P x ½R). Group.III Higher crosses of Patanwadi (6) (¼ P x ¾ M or ¼ P x ¾ R) (6). The study was conducted during Pre breeding (Feb-March) Breeding (April to 2nd week of June) Post breeding (2nd week of June to end of july).

The weekly semen samples of all the rams were collected by artificial vagina and volume and sperm concentration of ejeculate were recorded. In all a total of 450 collections were made. The weekly semen samples of all the six rams were pooled groupwise. A total of 75 pooled semen samples were used. The semen samples were centrifuged to remove the seminal plasma. In order to obtain spermatozoa free of seminal plasma, method described by Mann (1945) was used using Tris-buffer. In all three washings were given to the sperm pack present in the centrifuge tube. The washed sperm pack thus obtained, was transferred to a sterilized vial after breaking and dissolving it in tripple glass distilled water till a final volume of 10.0 ml was obtained.

The nucleic acid extracts were prepared using 10%. TCA and 95% ethanol using standard nucleic acid extraction procedure for biological fluids. Ribo-nucleic and Deoxy-ribonucleic acids in sperm cell pack were determined following the Orcinol and diphenyl amine methods described by Schneider (1945). Sodium salt of nucleic acids obtained from calf thymus gland (BDH- Biochemicals Ltd Polle, England) was used to prepare DNA standard whereas Ribonucleic acid (Sisco Research Lab Pvt. Ltd.,) was used for RNA standards. Nucleic acid extracts were processed and the readings were taken at 640 μ wavelength for RNA and at 600 μ wavelength for DNA. The nucleic acid standards were run parallel to the samples. The results were recorded in mg of nucleic acid per 10^9 spermatozoa

for sperm cell pack. Analysis of variance was conducted using two way classification with interaction as per Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

A. Deoxy ribonucleic acid (DNA). The DNA contens (mg per billion sperms) averaged 2.24 ± 0.24, 2.46 ± 0.10, 2.70 ± 0.21 in Patanwadi rams, Patanwadi helfbreds and higher crosses of Patanwadi rams, respectively. The present findings agrees with the findings of Eapen and Nasir (1964), Satter et.al., (1987) and Rao et.al., (1983). Similarly the DNA content (mg/10^9 sperms) averaged 2.48 ± 0.29, 2.50 ± 0.14 and 2.41 ± 0.19 during pre breeding, breeding and post breeding seasons, respectively.

The observed difference between the groups and between the seasons were found to be non-significant. These findings are in consonance with those of Rao et.al., (1983) and Mittal (1986). The interaction between groups and seasons was also found non–significants.

B. Ribo-nucleic Acid (RNA) : The RNA content (mg/10^9 sperms) averaged 0.74 ± 0.06, 0.70 ± 0.04 and 0.69 ± 0.05 in Patanwadi, Patanwadi half-breds and higher crosses of Patanwadi rams. During pre breeding, breeding and post breeding seasons the RNA content (mg/10^9 sperms) averaged 0.71 ± 0.06, 0.71 ± 0.04 and 0.70 ± 0.04 respectively.

The observed non-significant differences between the groups and between the seasons corroborates with the similar results obtained by Rao et.al., (1983) The interaction between groups and seasons was also found to be non-significant. It is concluded that DNA and RNA contents remained constant and was not affected by breed or season.

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RELATIONSHIP AMONG SEMEN PRODUCTION TRAITS IN JERSEY BULLS*

M.N. SUNDARA RAMAN, P. THANGARAJU and M. JOHN EDWIN

Department of Animal Genetics
Madras Veterinary College
Chennai 600 007.

Evaluation of semen production traits is helpful for assessing the semen production potential of bulls. To explore the possibility of using correlation among semen production traits as a tool for assessing the semen production potential of Jersey bulls, the present study was attempted.

MATERIALS AND METHODS

Data on semen characteristics of 6429 samples collected from 78 Jersey bulls, accrued over a period of four years in an organized stud farm at Udagamandalam, were utilized for the study. Ejaculate Volume (EV), Sperm Motility (SM) Sperm Concentration (SC) (for first and second ejaculate separately). Total Extended Volume (TEV) Pre-freeze Motility(PFM) Post-thaw Motility (PTM) and Total Doses of Frozen semen per collection (TDFS) were the semen production traits considered for the study. The correlation co-efficients among semen production traits were estimated as per Snedecor and Cochran (1967).

Part of the Ph.D thesis submitted by thefirst author in Tamil Nadu Veterinary andAnimal Sciences University, Chennai – 600 007.

RESULTS AND DISCUSSION

The first EV has a significant positive ($p \leq 0.05$) correlation with S.M.(0.208). Similar observations were noted by Sengar and Sharma (1965). Saxena and Tripathi (1980) and Tierney et.al., (1982) However, the positive correlation obtained in this study, between the second EV and SM (0.141) was not significant. The first EV was observed to have a low positive correlation with SC (0.09). This finding was akin to the reports of Gopalakrishna and Rao (1979), Tierney et.al., (1982). Patel (1985). Belrorkar (1986) and Bhavsar et.al., (1986) and Veerapandian (1982). However, the second EV had non-significant negative corelation with SC (-0.002). Findings of Sengar and Sharma (1965) and Saxena and Tripathi (1980) were also similar to the results obtained in this study. But the corelations recorded by these workers were significant. The correlations found between SM and SC were positive and significant ($P \leq 0.01$) for first ejaculate (0.278) and non-significant for second ejaculate. Positive significant correlation of SM and SC were also reported by Sengar and Sharma (1965). Saxena and Tripathi (1980), Tierney et.al., (1982),

Patel (1985), Mohanty and Dugwekar (1987) and Veerapandian (1992).

TEV had positive, non-significant correlation with PFM (0.235) and PTM (0.634). The relationship between TEV and TDFS was positive and significant (0.953). High correlation between TEV and TDFS values was due to the fact that, TDFS had direct linear relationship with TEV and the TDFS values were twice in magnitude of TEV as 0.5 ml capacity (medium) French straws were used for packaging the frozen semen. The correlation between PFM and PTM and between PFM and TDFS were positive and low, but non-significant. PTM was found to have a non-significant positive and low correlation with TDFS.

The correlation among semen production traits were generally low but non-significaant. From these findings it was found that most of the semen production traits were independent of each other and were influenced by other factors rather than their relationship with each other except between the traits TEV and TDFS. Therefore, the correlations among semen traits did not give any clear idea on use of their relationship to assessment of semen production potential of jersey bulls.

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SEROPREVALENCE OF CHLAMYDIAL AGENTS IN OVINE, CAPRINE AND BOVINE ABORTIONS

P. RAMASASTRY, N. MRUNALINI and M. RAMAKRISHNA RAO

Veterinary Biological Research Institute : Hyderabad

Chlamydial infections are widespread in nature and associated with pneumonia, entiritis, polyarthritis, encephlomalacia, conjunctivitis, abortions and genital infections of domestic animals. (Storz. 1971).

Seroprevalence of Chlamydial antibodies are reported in abortion cases of sheep, goats, cattle and buffaloes by many workers. (Dhingra et.al., 1979; Sharma and Baxi, 1983). Demonstrations of Chlamydial antibodies in sera from animals with history of abortions seem to be on the increase (Jain et.al., 1975; Krishna and Mathur, 1979 ; Dhingra et.al., 1980; Purohit and Gupta, 1983; Sharma et.al., 1983 : Khanna, 1984)

MATERIALS AND METHODS

In the year 1997, many Ovine and Caprine abortions were reported from many parts of Andhra Pradesh. The aborted animals were dull and anorectic. Abortions took place usually in last stages of pregnancy. The major changes were in fetal membranes. The affected placenta have shown various stages of necrosis. The tissue of membranes lost their natural colour, became tough and crumpled. The internal organs of fetus were congested.

One hundred two sera samples from the aborted Ovine, Caprine and incontact animals (Rams) were subjected for screening against seroprevalence of Chlamydial agents by AGPT after confirming them as negative for Brucellosis and Leptospirosis. Certain aborted Bovines that were negative for Brucellosis and Leptospirosis were also subjected for screening against Chlamydial agents.

Table depicting the samples screened and per cent positives.

Species	Status	Total No. Sera tested	Number Positive (%)
Ewes	Aborted	61	27 (44)
Goats	Aborted	28	12 (43)
She Buffaloes		8	4 (50)
Cows	Aborted	8	2 (25)
Rams	Aborted	13	8 (61)

The positive results were confirmed at Microbiology department, College of Veterinary Science, Palampur, Himachal Pradesh.

RESULTS AND DISCUSSION

Presence of Chlamydial antibodies in rams has to be noted, as rams do not appear to undergo natural infection (Buxton and Frazer, 1977). Aerosol transmission of the agents from infected rams may be an adding factor in quick spread of disease in a flock. In certain pockets of Karimnagar, Adilabad, Cuddapah and Krishna districts aborted large ruminants were positive for seroprevalence of Chlamydial antibodies where as Ovine and Caprine abortions were due to Chlamydial agents indicating the probable transmission from small ruminants to large animals.

In this communication the high incidence of abortions in Ovines and Caprines with the involvement of Bovines due to Chlamydia psittasi is placed on record which was not made earlier from this state.

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CLINICAL TRIAL WITH SOME ANTIMICROBIAL AGENTS IN REPEAT BREEDING CROSSBRED CATTLE*

S.K. BAISHYA, K.K. DAS, B.N. BORGOHAIN and H. RAHMAN [1]

Department of Vety, Gynaecology, Obstetrics & A.I.,
College of Vety. Science, Assam Agril. University,
Khanapara, Guwahati-781 022.

Repeat Breeding is one of the most important causes of infertility in cattle. The incidence was higher in crossbred cattle than in indigenous cattle of Assam (Deka et. al., 1980). The micro-organisms present in the genital tract play a significant role in the failure of conception/or early embryonic death resulting in repeat breeding. To combat the infection, clinical trial with different antimicrobial agents was carried out in 36 repeat breeding crossbred cattle.

MATERIALS AND METHODS

The clinical trial was carried out in 36 repeat breeding crossbred cattle of different private farms in and around greater Guwahati. The animals included in the present study had normal oestrous cycle, estrus period and apparently normal genitalia but failed to conceive from 3 successive inseminations with semen of know fertile bull. All the 36 animals were divided into 6 groups and administered one of the following drugs.-

Group. A: Treated with Myron tabs; Dose – 10 tabs twice daily for 3 week orally from the day of estrus.

Group.B: Treated with Oriprim-U bolus; Dose – 2 boli dissolved in 40 ml distilled water I.U. for 2 days during estrus period.

Group. C: Treated with Lugol's iodine (0.25%) Dose – 40 ml I.U. for 2 days during estrus period.

Group. D: Treated with Gentamicin; Dose – 10 ml mixed (50mg) in 30 ml distilled water I.U. for 2 days during estrus period.

Group. E: Control group (forB,C and D) was given 40 ml distilled water for 2 days during estrus period.

Group. F: Control group (for group A) was given no treatment.

Artificial insemination was carried out at the next estrus both in treated and control groups with good quality of frozen semen. Artificial insemination was repeated upto 3rd estrus whenever animals returned to heat. Pregnancy diagnosis was carried out between 60 and 90 days after last A.I. by rectal examination. Statistical analysis of the date was done as per Snedecor and Cochran (1968).

Part of M.V.Sc.thesis submitted to AAU by the first author.

1. Associate professor, Department of Microbiology.

RESULTS AND DISCUSSION

The conception rate was found to be highest in those treated with Gentamicin (66.67%) followed by Oriprim-U bolus (50.00%) Myron tablets (50.00%) and Lugol's iodine (33.33%). In the two control groups the conception rate were 16.67 per cent in each. However, the difference in conception rates were statistically nonsignificant among different groups. This finding was in close conformity with the observations recorded by earlier workers (Murthy and Rao , 1978, Venkateswarlu et.al., 1983). It was evident that higher percentage of conception rate was recorded in Gentamicin treated group (Group D) which might be attributed to broad spectrum antimicrobial action of Gentamicin and due to infrequent use of this drug in this region for the routine treatment of genital infection.

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MONSTERS IN GOATS

K.G.S. RAJU, K.S. RAO, M.R. REDDY, V.S.C. REDDY, and G.P. SHARMA

Department of Animal Reproduction, Gynaecology and Obstetrics,
College of Veterinary Science,
Rajendranagar, Hyderabad.30

Craig (1959) and Roberts (1971) have dealt in detail about monstrosities leading to dystocis in domestic animals. The incidence of monsters like embryonic duplications and schistosomus reflexus is observed to occur commonly in cattle and rarely in sheep and goats (Roberts,1971). Amongst double monsters, the duplication of caudal portion is rare. Jones and Hunt (1983) opined that malformations are due to abnormal duplication of the germinal area giving rise to fetuses whose body structures are partially duplicated. The present paper is to place on the record the incidence of two monocephalus-dipygus and one schistosomus reflexus monsters in local goats. (Figs 1-3),

Monocephalus – Dipygus Monsters: These monsters were encountered during the routine abattoir survey. The two monsters present the normal feature upto the thorecic region. The spinal cord was found to be duplicated from thoraco-lumbar region with distinct duplication of abdominal and pelvic regions. An extra pair of fore limbs were attached to the dorsal aspect of the thorax of the fetuses. But in one monster, the tongue was found to be duplicated. Similar type of double monster was reported by Doijode et.al., (1992) in a sheep.

Schistosomus Reflexus : The fetal monster revealed a marked ventral curvature of the spine. The occiput of the head lied near the sacrum. There was severe closure defect of thorax and abdominal cavities with exposure of viscera. The liver was found to be enlarged. All the four limbs were ankylosed. Similar findings were reported by Nanda et.al., (1983), Sharma et.al.,(1988) and Balaswamy and Narshimha Rao (1997) in goats. Besides these observations, the monster exhibited a split in the upper lip (hare lip) and ears were short and stumpy

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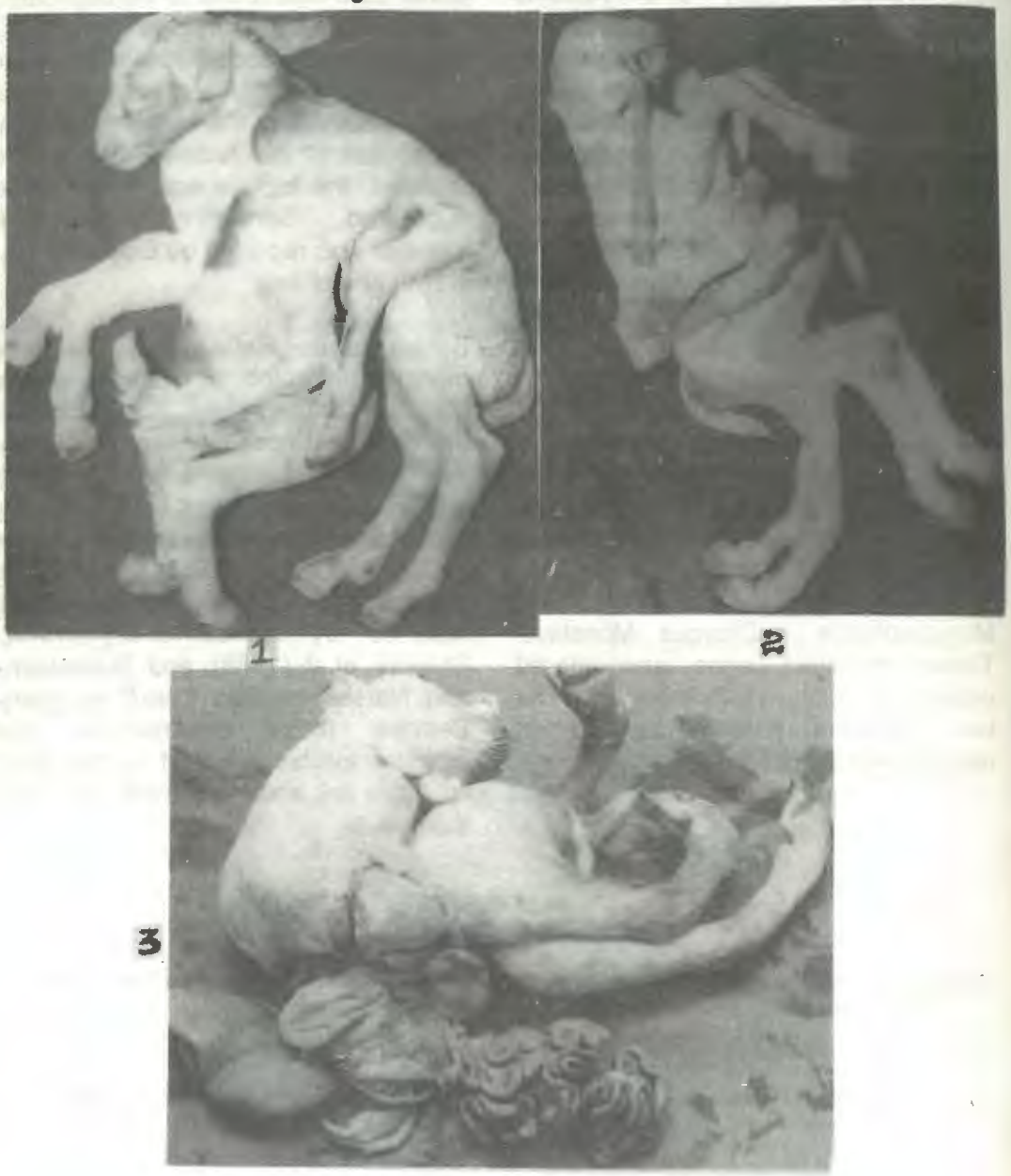
Fig. 1 & 2 : Monocephalus – Dipygus Monsters
Fig. 3 : Schistosomus reflexus



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DICEPHALUS TETRAPUS TETRABRACHIUS MONSTER IN MURRAH GRADED BUFFALO

SURESH KUMAR, SHIV PRASAD, UTSAV SHARMA,
RAJU SHARDA [1], Y.P.S DABAS [2] and S. N. MAURYA

Department of Gynaecology and Obstetrics
College of Veterinary Sciences
G.B.Pant University of Agric. & Tech.Pantnagar – 263 145, Distt. U.S Nagar, U.P.

Only limited reports are available on conjoint twins with varying degree of duplication (Velhankar et.al., 1968; Sahu, 1968; Dabas et.al., 1995). Information about such cases in buffaloes are still scanty hence this case is placed on record.

A buffalo was presented in the College Clinics with the history of labour pain since last 12-13 hours. Though forelimbs and head of the foetus were available in the birth canal, parturition could not proceed. Local manipulations proved futile. Per vaginum examination revealed anterio-dorsal presentation of foetus with two heads and excessively large sized thorax. The conceptus, a conjoined dicephalus tetrapus tetrabrachius monster attached to placenta through a single umblicus was delevered by caesarean section under local anaesthesia adopting standard surgical procedure. The monster appeared to have been dead for less than 12 hrs.

The detailed examination of the monster revealed a duplication of all body parts except heart and lung which were encased in a common thoracic cavity. Each conjoined foetus had a separate abdominal cavity; a pair of hind and forelimbs, ovaries, kidneys; one each of head, tail, vertebral column, spleen, liver and complete G.I. tract. The reproductive tract (oviducts, uterus, cervix and vagina) was ill developed with external genetalia being represented by a slit of one centimeter length. Anal opening in each of the conjoined foeti was patent. There was no anatomical defect in heads of the foeti.

1.Department of Surgery & Radiology 2. Veterinary Clinics.

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using Folly's catheter with Dulbecco's modified phosphate buffered saline (Hi-Media), however failed to enter into the uterine horn due to adhesion and evacuate the larger bone pieces.

After the onset of winter the animal became anoestrus with closed cervix and the bone mass became compact in the affected uterine horn. The necropsy revealed thickening of the uterine wall with slight bulging of the right uterine horn and three intact bone (Scapula, femur and metatarsus) with white gelatinous pus with complete adhesion in the body and uterine horn.

Bacteriological examination of the uterine discharge, revealed presence of Bacillus species in all three samples with fungal contamination with Aspergillous species.

The cause of abortion and maceration of foetus was perhaps due to bacterial or fungal infection. In the macerated case only three intact bone was found which may be the remnants after expulsion and/or adsorption of the foetal bone due to long-standing foetal maceration.

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