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Dimensions of Infertility And Sterility In Cattle And Buffaloes*

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INTRODUCTION

Livestock constitutes an integral part of the agrarian economy in India not only by supplying valuable animal proteins for the ever increasing human population through milk, meat and eggs, but also by contributing vital motive power so essential for agricultural operations, besides providing organic-manure to enhance soil fertility. The contribution of livestock to Gross National Product (GNP) is phenomenal - around Rs.200,000m.per annum, 80% of which is from cattle and buffaloes. Livestock have a much large contribution to manpower employment constituting a vital element in Rural Development Programmes.

Dairy Cattle/Livestock production has helped the farmers/cultivators through the availability of fluid cash to meet with their routine needs of seed, fertilizer purchases etc. Milk with a contribution of Rs. 100,000 m. to the GNP comes next to rice with Rs. 120,000 m. contribution.

As per 1982 census, India possesses 184.86 m. cattle, of which approx. 10 m.are cross-breds and 61.06 m. are buffaloes, comprising 50% of livestock population of Asia. The contribution of buffalo milk is very significant in comparison to Asia (68.06%) as well as the world (65.05%), even though less attention is paid to buffalo development in India (Anon, 1986).

Cattle cross-breeding programme has definitely made an impact on rural economy in selected areas, not only by enhanced milk production, but also by providing opportunities for additional employment to both under- employed and unemployed rural families including school drop-outs, possessing at least minimum asset holding for participation in the programme.

The success of Dairy cattle and buffalo economics lies in ensuring proper and optimal reproductive rhythm of each individual cow and buffalo in the herd, within normal physiological range. Any deviation or prolongation in the breeding rhythm results in a progressive economic loss due to widening of dry period, reduced calvings and lactations during the life span of the animal. Barren or infertile cows/buffaloes mean a direct loss in milk production, whereas reduced calf crops hamper the selection efficiency in long term Dairy herd improvement.

Thus, fertility of milch animals plays a vital, pivotal role in dairy economics. Efforts should therfore be made to enhance fertility in dairy animals by narrowing their dry period to the barest minimal range of 60 to 90 days. (Kaikini, 1989).

Incidence of Infertility

In a quick All India Survey of Bovine Infertility covering 20,000 cattle and buffaloes in organised and rural sectors conducted by

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IVRI Team, 3% animals were diagnosed to be sterile and 8 to 10% sub-fertile, with an overall 7.70% incidence of infertility (Bhattacharya, 1954). Rao (1954) in his survey of bovine infertility covering 10,865 bovines in India, recorded a high incidence of infantile genitalia (18.99%) and sub-active gonads in 47.87% cows and 54.4% heifers.

In an intensive two year (1952-54) State wide survey on Bovine infertility conducted by him covering over 10,000 cows and buffaloes covering over 45 cattle breeding farms, buffalo farms, goshalas and rural areas in the erstwhile Bombay State, Kaikini(1967) recorded an incidence of 4% sterility and 8% sub-fertility. A team of F.A.O. experts examined 3,000 cows and buffaloes comprising urban and rural animals in India and found that 61.8% cows and 31% buffaloes had sub-active ovaries (Lagerlof, 1954).

By and large, the rural bovine population in India is of 'Non-Descript' (ND) type, with an assorted poor genetic make up. Such heifers not only mature remarkably late, but even their effective breeding period is distressingly irregular with resultant low number of calves born per cow, combined with poor total life time milk production. Obtaining cross-bred progeny by A. I. from ND cows is also directly proportional to their erratic reproductive efficiency. This is allied to recovering metal from scrap fed in Mini Steel Plants. As the generation interval is increased, the rate of genetic gain is slower, jeopardising the economic interests (Kaikini, 1975).

Dimensions of Infertility And Sterility

The technical dimensions of infertility and sterility are very many extending from genito-gonadal anomalies, gynaeco-pathological conditions and Repeat Breeding Syndrome (RBS) to infectious Sexually Transmitted Diseases (coital infections STD). Besides these, particularly in high yielding animals such as the cross-breds, stress factors play a major role.

Protein deficient diet, malnutrition including Vitamin A deficiency, minerals and trace elements deficiency, excessive heat and cold/climatic stress, parasitism, high milk yield, acetonemia, ticks, infections diseases and hoof troubles lead to stress with resultant disturbed breeding and rhythm of milch cattle and buffaloes.

The main contributing factors responsible for a good fertility index are : a sound Central Nervous System (CNS) ; optimum endocrine balance between the gonadotrophins and sex hormones combined with the proper response of the individual sexual apparatus of the animal. The term 'Endocrine Constitution' indicates the inter-relationship between these factors. Oestrus rhythm is largely controlled by the endocrine constitution of each cow (Lagerlof, 1951). There is an obvious distinct difference in oestrus manifestation in various breeds of cattle (Luktuke, 1982).

Measure of Fertility

The measure of fertility should be based on the number of first services (A.I.or natural) and actual conceptions diagnosed by gynaeco-clinical examination, since non-return (NR) rate is meaning-less under our Indian conditions. Farmer is not careful to watch the cows/ buffaloes for appearance of subsequent heats. He is under the erroneous impression that once inseminated, the cow/buffalo must be pregnant.

Genital/Gonadal Anomalies

The conditions that lead to sterility or permanent infertility are genital anomalies such as developmental defects of mullerian duct leading to various anomalies of the vagina,

cervix and uterus such as uterus unicornis, absence of cervix, cervix duplex, segmental aplasia of salphinx and didelphic uterus.

Single autosomal recessive gene is responsible for causing hypoplasia or under-development of testes and ovaries. In this condition one or both gonads are small, narrow and functionless. Lagerlof (1939) recorded a high incidence (17.5%) of ovarian hypoplasia in Swedish Highland Cattle (SKB). Cows with both ovaries hypoplastic were infertile with absence of oestrous cycle. The incidence of gonadal hypoplasia was reduced by adopting a nation-wide rigorous sexual health control programme and culling cases of uni or bilateral and partial or complete gonadal hypoplasia (Lagerlof and Boyd, 1953).

Gilmore (1952) listed lethal factors in cattle such as Achondroplasia, amputated calves and congenital dropsy, which are of genetic origin due to homozygosity of recesssive genes, mostly due to inbreeding. Great care should be exercised in formulating and monitoring breeding policy while using frozen semen for A. I.

Functional Disorders

Aberrations of reproductive functions occur on account of some endocrinological abnormality which is difficult to pin-point or detect even with the modern hormone assay techniques. Abnormalities occur due to weak endocrine constitution, nutritional deficiencies, stress of production and climatic/ environmental stress. The major functional disorders are : (1) Anoestrus, (2) Suboestrus, (3) Anovular heat, (4) Delayed Ovulation and (5) Cystic Ovarian Degeneration (COD).

True Anoestrum

This condition is due to quiescent ovaries with absence of cyclical activity. It results due to insufficient release or production of gonadotrophins to cause folliculogenesis. Failure of ovarian response also results in anoestrus.

In true anoestrum cases, the cow/buffalo is empty (non-pregnant) with smooth and oval or round ovaries giving no palpable evidence of either follicular or luteal activity.

Post-partum anoestrum is more acute in buffaloes than in cows. The post-partum fertile oestrus interval and inter-calving period is considerably wide in buffaloes than in cows. According to Bansal (1976), anoestrum accounted for 57 to 70% of reproductive disorders in buffaloes.

El-Hariri *et al* (1980) noted increased fertility in stall fed buffalo heifers fed with ration supplemented with 20g. milk powder plus 1 g. pot. iodide and 100g. iodine every alternate day. 90% of experimental heifers manifested oestrus with 80% CR. Bhatia et al (1984) found beneficial effect of dicalcium phosphate supplementation on anoestrum in buffalo cows. 60% of the cases exhibited heat, of which 50% conceived.

Clomiphene Citrate : Kaikini *et al* (1977) found Fertivet-FVT300 (Ar-Ex Labs. Bombay) therapy useful for activating anoestrus gonads (80%) in ND cows with resultant ovulations. Deshpande *et al* (1976) reported encouraging results (73.33%) of fertivet therapy for inducing heat in anoestrous buffaloes. Hukeri *et al* (1979) found Fertivet oral therapy effective to induce oestrus in 85.27% of the treated group, with manifestation of heat in 11.13 days (AV). Kodagali *et al* (1983) found intra-abomasal injection of Fertivet 600 to 800 mg. to be 100% effective for oestrus induction with ovulation in anoestrous buffaloes. Pattabiraman *et al* (1983) found intra-vaginal administration of Fertivet comparable to oral therapy. Aminuddin and Tanwar (1988) in

buffaloes and Reddy *et al.* (1990) in cows reported beneficial effects of Clomiphene therapy for anoestrum.

Prajana Therapy: Rao and Keshavamurthy (1971) reported efficacy of Prajana (Indian Herbs, Bangalore) therapy for anoestrum in buffalo cows and heifers, with induced oestrus in 84.78% cases with CR of 72.72%. Porwal *et al* (1976) reported superiority of Prajana therapy to painting Os uteri with Lugol's iodine and Supermindif supplementation.

Deshpande (1983) reported Aloes Compound (Alarsin, Bombay) oral therapy suitable as ovarian activator in anoestrus buffaloes.

According to Bulman and Laming (1978), some cows (5.2%) resume ovarian cyclical activity within few days of calving and then become anoestrus. True anoestrum is frequently diagnosed in high yielding dairy cows, first calf heifers and suckler cows. Anterior pituitary appears to be refractory to stimulation with GnRH in the immediate post-partum period, probably due to progesterone induced negative feedback (Laming, 1987). Prolactin inhibitory factor (PIF) secretion by hypothalamus is low if prolactin secretion is high. This suppresses GnRH secretion and resultant gonadotrophin production.

I. M. injection of PMSG 3000-4500 I. U. helps in inducing/stimulating ovarian activity. Likewise, single dose of GnRH 0.5mg. I. M. is effective therapy. Progesterone with Oestrogen; PRID insertion for 10 days; cervix infusion with 20 ml.1% Lugol's iodine; painting of cervix with Lugol's iodine and Clomiphene citrate therapy have been reported to be beneficial in inducing oestrus in cows and buffaloes. Their efficacy is due to stimulation of short luteal phase that normally precedes the first normal oestrus cycle in post-partum cases.

Synthetic Analogues of Progesterone Hormone: Progesterone hormone is available as synthetic analogue called 'Progestogens' which are equally potent when administered by feeding, daily I.M. injections or subcut /intra-vaginal insertion of long acting (Depot) preparations in the cow/buffalo.

Commonly available Progesterone Preparations are : 1. Methyl acetoxy progesterone (MAP) which is less potent. Dose: 180-200 mg. per day in feed for 15-18 days.

2. 6 Chlor-dehydro-17 acetoxy progesterone (CAP) or chlormedinone. Dose: 10 mg. in feed for 15-18 days.

3. Melengesterol acetate (MGA) is a highly potent preparation. Dose: 1mg. per day in feed for 15 days.

4. Progesterone I.M. injection 25 mg. daily for 10 days.

5. Progestogen Releasing Intravaginal Device (PRID): These are silastic coils impregnated with 1.55g. progestogen with a gelatin capsule of 10 mg. estradiol benzoate attached on the inner surface. It provides a source (Depot) of progesterone and estradiol which get slowly absorbed through vaginal mucosa. The coil is kept in anterior part of vagina of the cow/buffalo for 10 days and then removed. Sudden withdrawal of progesterone source (coil) causes sharpdecline/drop in progesterone blood level with sequential events bringing the cows in heat. Insemination be done at 48 and 72 hours post withdrawal of the coil. This is popularly termed 'Appointment Breeding' with oestrus 'made to order'.

6. Combined Therapy: PRID is inserted in vagina. On 8th day of insertion, administer

PGF2 alpha 25mg. and PMSG 400 I.U. intramuscularly. PRID is removed on day 10. Cows are inseminated on Day 12 and Day 13. 100% occurrance of heat with 55 to 62% CR have been reported in cows and heifers by Lokhande *et al* (1983, 1984) and Bhosrekar *et al* (1986). Similar findings are reported in buffaloes by Rao (1982), Rao and Rao (1977, 1978, 1979a, 1983), Narasimha Rao *et al* (1985, 1987) and Narasimha Rao and Sreemannarayana (1983).

GnRH (Receptal-Hoechst) has been successfully used in cows and buffaloes for induction of oestrus and treatment of repeat breeding due to delayed ovulation or anovular heats (Rao and Rao, 1979a; Bhosrekar *et al*, 1986 and Muzumdar, 1989).

Sub-Oestrus (Silent heat)

Sub-oestrus is the condition in which the cow/buffalo has normal cyclical activity (detectable on gynaeco-clinical examination), but behavioural signs are not normal (sub-normal) and indistinct. Silent heat (Sub-oestrus) is common in buffaloes (20 to 40%) with normal cyclical changes and unobserved oestrus. Various hormonal therapies have been used for treating sub-oestrus in buffaloes, such as Estrumate, ICI (Rao and Rao, 1978, 1979a; Chede, 1989) with encouraging results.

A genetic predisposition to silent heat has been identified with certain sire lines showing a statistically significant effect (Labhshetwar *et al*, 1963). PGF_2 alpha therapy followed by fixed time insemination (appointment breeding) is useful.

Anovular Heats

In our studies based on 500 local (ND) cows of University Heifer Project at Borgaon (Akola), anovular heats were detected and recorded in 24% of the animals that came in heat resulting in repeat breeding (Kaikini, 1975). Similar findings have been reported by Khan and Luktuke (1967) and Deshpande and Deopurkar (1981). According to Van Rensburg (1962), many of the early post-partum heats are accompanied by a high incidence of anovular heats (34%)or delayed ovulations (66%) which is due to inadequate LH level. For cases of anovular heats, GnRH treatment is indicated on Day 13 of oestrus cycle and A.I. on observed heat with good results (Bhosrekar *et al*, 1986).

Delayed Ovulations

Delayed ovulations generally occur in cyclical non-breeder infertile cows. It is due to delayed LH surge. GnRH injection I.M. at the time of AI/service is indicated with resultant ovulation within 24 hours.

Cystic Ovarian Degeneration (COD)

Ovaries are cystic when they contain one or more (multiple) larger follicles 2 to 3 cm. in diameter. Cysts are due to anovulations. Follicles instead of getting atretic, continue to increase in size and persist, enlarging the ovary. Cow becomes Nymphomaniac. Stress of milk production/yield results in cystic ovaries (Hendrikson, 1956). An incidence of 7-8% cystic ovaries is reported in Tharparkar, Sahiwal and Gir cows (Bhosrekar, 1973).

Follicular cysts can be treated by HCG (LH) and GnRH. A dose of 3000 to 4500 IU of HCG is recommended. Mucometra develops in untreated cases of COD.

Persistent Corpus Luteum (PCL)

Persistent C.L. (PCL) is essentially due to uterine lesions. Sequential gynaeco-clinical examination is essential for diagnosing PCL cases. Corpus luteum remains in the ovary exerting inhibitory effect on the anterior pituitary gland.

Prostaglandin therapy is best indicated in PCL cases. Prostaglandins PGF_2 alpha (Lutalase Hoechst; Dinoprost Upjohn; Dinofertin Alved) and their analogues of ICI and Searle companies (Estrumate; ICL-80996; Synchromate; Cloprostenol) have shown luteolytic effect with satisfactory post-treatment fertility. These are effective only on CL of Day-5 and above (Chatterjee *et al*, 1989).

Sexually Transmitted Diseases (STD)

Brucellosis, Campylobacteriosis (Bovine Genital Vibriosis) and Trichomoniasis are dreaded diseases causing abortions and Early Embryonic Mortality (EEM). These can be prevented by adopting A.I. with semen of clean bulls. Emerging diseases are : Leptospiral abortions; Mycotic abortions and IBR-IPV abortions. These diseases are categorised under "Infectious Infertility".

Management For Fertility Improvement

1. It is imperative that regular, systematic and well planned sexual health control programme with periodical gynaeco-clinical examination and treatment of detected cases, be implemented with proper recording of individual Life History Cards for female animals. This should be rigidly followed along with practice of A.I.

2. Regular monitoring of field cases is a must since casual diagnosis and therapy only once at the clinic or Animal Health/P.D. & Sterility camps will not serve any purpose. Follow up is most essential.

3. Improper and negligent managemental regime is a major contributory factor of infertility in cows & buffaloes. Optimum managemental practices go a long way in reducing stress conditions and help in maintaining good fertility.

4. It is necessary to educate the farmers and livestock owners regarding the importance of breeding hygiene, prophylaxis, heat detection, periodical gynaeco-clinical check up and maintenance of records of their cows and buffaloes.

5. In view of our long and mature experience in this field of specialisation, the following suggestions are made in the best interests of State Departments of Animal Husbandry:

(a) Subject Matter specialists in Gynaecology at Taluka level be appointed. Similar positions exist for Crop Sciences /Horticulture in Agriculture/Horticulture Depts.

(b) Govt. Veterinary Polyclinics be suitably strengthened/fortified by creating a post of Veterinary Gynaecologist in each Polyclinic.

These suggestions if implemented will go a long way in tackling fertility problems in a comprehensive manner.

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Embryo Transfer Technology In Buffaloes*

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SUMMARY

During the past years, through systematic scientific investigations and innovations, there is a steady progress in terms of superovulatory responses, embryo recovery and pregnancy rate following embryo transfer among buffaloes. From the early recovery rate of 0.6 transferable embryos currently 2.0-2.4 transferable embryos are recoverable in different farms with altered superovulation protocols. Folltropin and Super-Ov among many compounds have comparatively given better results both in terms of follicles developed and ovulated and embryos recovered. The lower number of follicular population and poor follicular development, as revealed through real time ultrasound scanning, explains the lower ovulatory response. The growth rate of the largest follicle and time of ovulation post PGF has also shown great variability in animals. Endocrine studies suggest that unovulated follicles contribute massive quantities of estrogen along with progesterone. High prolactin levels in buffaloes during summer months are also associated with poor follicular maturation. The birth of calves using *in vitro* fertilization technology and frozen thawed embryos are the recent milestones achieved in this technology. Inspite of some problems related to buffalo embryo transfer, the basic embryo transfer techinque has been adopted with success resulting in over 100 pregnancies/calves.

INTRODUCTION

In animal populations, individuals differ widely in their lifetime reproductive success

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(Brown,1988) the major components of which are survival to breeding age, reproductive life span, average fecundity per year and offspring survival between birth and reproductive age, effected grossly by the micro environment, ecological and socioeconomic factors. Buffalo as a domestic animal with its predominant home tract in South East Asia and Mediterrian, happens to be a unique animal whose capabilities as living tractor, milk machine, meat producer and an integrator of man-crop-livestock farming system have found great acceptance in the rural economy of Asia and Africa (Madan,1990).

Considering the current economic demands for buffalo milk and meat, a strong tendency has developed among buffalo breeders to aim at breeding a triple purpose type with high milk yield, draught ability and reasonable growth rate on roughage based feeding. Cross breeding programmes and selection for economic traits are putting extra premium for milk yield and may thus exert unfavourable genetic pressures in population ranges against their respective superiority, be it a Swamp or Riverine buffalo. The change in breeding goals necessitates, on one hand, fast multiplication of a high yielding animal and developing a high milk producing breed, it simultaneously also implies ensuring the preservation of germplasm for the conservation of basic genetic resource. This can be achieved through the availability of embryo transfer technology based programs like Open Nucleus herd with Multiple Ovulation and Embryo Transfer.

The embryo transfer (ET) program in Buffaloes is of recent origin. The intitial success of Drost *et al* (1983) in U.S.A., who pioneered application of the technology to buffaloes was soon followed with successful birth of buffalo calves in Bulgaria, (Vlakhov *et al* 1985) and India (Misra 1988, Madan *et al*., 1989, Singh *et al*., 1988). Studies on super-ovulation among buffaloes have also been carried out in Thailand (Thungtanawat *et al*. 1981; Nityawardana *et al* 1982; Parnpai *et al*., 1985 ; Chantaraprateep *et al* 1989a, 1989b; Techakumphu *et al*., 1989; Venitkul ,1989) Bulgaria (Karaivonov *et al* 1987; Alexiev 1990), Malaysia (Jainudeen 1989) Pakistan (Mehmood *et al* 1989; Rahil *et al* 1989) and India (Madan 1984, Madan *et al* 1988; Singla and Madan 1988; Madan 1990; Madan *et al* 1990; Taneja *et al*., 1990; Singh and Madan 1990; Singla and Madan 1990a, 1990b; Tuyen *et al* 1990; Madan *et al* 1991; Misra *et al* 1990, 1991; Singla *et al* 1992).

Buffalo reproduction is known to suffer with inherent problems like late maturity, poor estrus expressivity particularly in summer months, long post-partum intervals and low conception rates (Madan 1984, 1985). Fertility performance and reproductive efficiency among buffaloes under tropical conditions has been investigated by Madan and Raina (1984) and the data shows distinct climatic/seasonal effects on reproductive behaviour of this animal. In recent years, considerable attention has been focussed in the field of reproductive endocrinology as a mean to identify specific problem and to adopt measures for augmenting reproduction. The basic information available on reproductive parameters suggests that this animal has certain peculiarities which need full understanding to exploit the productive potential of this animal.

The peculiarities among these animals are both at the hypophysial as well as at gonadal levels, as suggested not only by the intrinsic endocrine rhythm shown by the animals but also due to the varying responses which these animals show towards exogenous administra-

tion of hormones. In studies conducted by us on buffalo endocrinoloy, simultaneous to similar investigations among Zebu/zebu crossbreds (Palta and Madan, 1984; Suri and Madan, 1984; Prakash and Madan, 1985; Khurana and Madan, 1986a, 1986b, Madan *et al* 1990 and Madan, 1984) are indicative enough that the hormone rhythm in this species and the quantitative relation of discrete reproductive events to endocrine levels are at variance. A number of other factors have also been identified to be responsible for poor responses among buffaloes (Madan 1990). Under these circumstances, it is logical to expect that the animal responses during the process of synchronization and superovulation intended to be induced as a result of exogenous hormone administration will not have predicted responses as would have been expected in cattle.

Superovulatory responses among buffaloes : The initial comparative response among the buffaloes using different hormone prepara- tion at dose levels similar to those tried for cattle is given in Table 1. FSH-P, Super Ov and Folltropin were administered at a dose level of 32mg, 75 units and 30ml equivalent to 24 PGF in a four day schedule. The total transferable embryos varied between 0.15 to 0.94 embryos per flush in this trial. Super Ov gave a high percentage of

Table 1 : Superovulatory responses in buffaloes

Drug	No. of Animals	Total Ovulations	Total Embryos	Transferable Embryos	Degenerated Embryos	Unfertilized Ova
FSH – P	42	3.17 ± 0.89	0.95 ± 0.24	0.55 ± 0.17	0.07 ± 0.04	0.33 ± 0.12
Super–Ov	13	3.38 ± 0.28	0.85 ± 0.30	0.15 ± 0.15	0.23 ± 0.17	0.46 ± 0.27
Folltropin	32	2.56 ± 0.31	1.63 ± 0.34	0.94 ± 0.28	0.31 ± 0.16	0.38 ± 0.13

Table 2 : Superovulatory responses in buffaloes

Drug	No. of Animals	Total Ovulations	Total Embryos	Transferable Embryos	Degenerated Embryos	Unfertilized Ova
FSH – P (Priming)	13	39 (3.00)	28 (2.66)	15 (1.15)	2 (0.15)	11 (0.85)
Folltropin	11	33 (3.00)	25 (2.27)	18 (1.64)	2 (0.18)	5 (0.45)

Table 3 : Superovulatory results from responding animals

Drug	No. of Animals	Total Ovulations	Total Embryos	Transferable Embryos	Degenerated Embryos	Unfertilized Ova
FSH – P	10	36 (3.60)	28 (2.80)	18 (1.8)	–	5 (0.5)
Folltropin	22	72 (3.27)	58 (2.63)	41 (1.86)	6 (0.27)	9 (0.40)

degenerated and unfertilized ova. However, the collection procedure and technique at this stage of our initial work on embryo transfer was similar to cows which might have contributed to low returns. In another study (Table 2) a priming dose of FSH-P was administered on day 3-4 of the cycle (5mg) and then a regular dose protocol was followed. FSH-P gave 15 (13 animals) and Folltropin gave 18 (11 animals) transferable embryos with an average of 1.15 and 1.64 embryos per flush. Total Embryos per flush (including unfertilized and degenerated) collected were 2.66 and 2.27 respectively. This has been observed that among the selected donors there is a high percentage of animals (18%) which do not respond to superovulatory treatment even when animals were specifically synchronized for this treatment after selection. Considering the data obtained from responded only those animals which responded with 2 or more ovulations, the picture among buffaloes shows improvement (Table 3) The mean response of transferable embryos for both the hormonal preparations is around 1.8 embryos. Progesterone assay data on the poor responding animals suggest that prostaglandins inspite of being used at a 2 dose combination at 12hr interval at the time of superovulation is not able to bring a synchronized estrus among all the superovulated animals and hence we have a higher percentage of unfertilized and degenerated embryos. Real time ultrasound scanning for the study of follicular population on the day of estrus and ovulation in buffaloes (Manik *et al* 1992) has also shown that ovulation time in response to PGF treatment varied considerably among animals. Since Folltropin showed superior responses another experimental trial (Table 4) was held in which the superovulating compound was administered in a constant dose, gradual descending dose and steep descending dose (Total 30 ml Folltropin, 12 hour interval for 5 days). Though the ovulation rate was similar in all the three groups, total embryos recovered and transferable embryos were more in the gradual descending group than the other two groups (Table 4).

Using the same superovulatory compound in Zebu (Sahiwal) and crossbred population (Friesian and Brown Swiss Crosses, Table 5) an average of 5.10, 6.76 and 4.46 embryos could be obtained from Friesian (KF), Brown Swiss (KS) and Sahiwal (Sw) animals out of which 2.89, 2.76 and 2.85 embryos were transferable. The response in terms of transferable embryos was maximum among the Freisian crosses but there were no significant differences in the response pattern either in terms of hormone preparations or the breeds tested. However, Super-Ov which had given

Table 4 : **Superovulation response in buffaloes (Bubalus bubalis) to different treatment regimes of Folltropin**

Dose schedule	No. of animals	Mean ± SE		
		No. of CL	Embryos recovered	Embryos transferable
Constant dose	10	2.50 ± 0.48	1.20 ± 0.53	0.50 ± 0.27
Grandual descending dose	16	2.75 ± 0.45	2.13 ± 0.55	1.31 ± 0.51
Steep descending dose	6	2.17 ± 0.83	1.00 ± 0.52	0.67 ± 0.33

poor results among the buffaloes gave comparable results to FSH-P and Folltropin in the cattle. These results are also indicative of the fact that embryos collection was optimal for cattle and mean response was comparable to figure obtained in countries wher the technology has been well established.

Embryo Transfer results at other centres : There are other centres in India which during the past couple of years have published data on superovulatory responses among buffalo using different hormonal preparations. The salient feature of the results obtained are summarized below.

Under the national Technology Mission programme of the Govt. of India, considerable success has been achieved in the application of ET technology among buffaloes, the overall results of which are summarized (Kurup, 1991) below.

Table 5 : **Superovulation responses among cattle**

Breed/Cattle	Drug	Animal Yielding Embryos		
		No. of animals	Total embryos	Transferable embryos
KF	Super Ov	20	117	59
	Folltropin	8	34	27
	FSH–P	10	43	24
	Total	38	194 (5.10)	110 (2.89)
KS	Super Ov	15	120	43
	Folltropin	8	71	26
	FSH–P	15	66	36
	Total	38	257 (6.76)	105 (2.76)
SW	Folligon	4	15	10
	Super Ov	4	11	10
	FSH–P	5	32	17
	Total	13	58 (4.46)	37 (2.85)
All Breeds Grand Total		89	509 (5.72)	252 (2.83)

Table 6 : **Superovulatory responses to different preparations**
(Figures in parenthesis show average)

India: SAG (Gujarat) Mishra *et al*(1991)	Donors	Animals Flushed	Embryos		
			Recovered	Transferable	Unfertilized and Degenerated
FSH–P	151	137	169 (1.2)	104 (0.8)	45 (0.3)
PMS	70	59	19 (0.7)	15 (0.6)	
Folligon	69	65	184 (2.7)	100 (1.5)	84 (1.1)
CIRB (Hissar) Jain *et al*					
FSH–P	98	59	54 (1.1)	–	4 (0.2)
FSH–E	17	11	2	–	–

Table 7 : Embryo Transfer Technology (Buffalo)

Donors Responded (%)	28-87
Animals Flushed	991
Embryos Recovered	1881
Embryos Per Flush	1.90
Transferable Embryos	1026
Transferable Embryos Per Flush	1.40
Embryos Transferred	766
Pregnancies	106

Fig. 1 : First Ever Buffalo IVF Calf (Pratham) With its Surrogate Mother (Recipient Buffalo)

Single ovulation and embryo transfer in buffaloes

Because of poor superovulatory responses among buffaloes to superovulatory compounds noted by most workers who attempted embryo transfer programme at different centers,a study was taken up to recover single embryos after natural estrus and artificial insemination procedures. The embryos were recovered by nonsurgical flushing of a single horn in each animal either on 5th or 6th day post estrus. The fresh embryos were transferred within 4 hours of recovery to the recipients in the ipsilateral horn (Singla and Madan, 1990). Out of 69 attempts for embryo recoveries (Table 8), 69.6% embryos were located and recovered. The stages of the embryos ranged from 16 cell to expanded blastocyst. Out of 48 embryos recovered, 22 (45.8%) were of transferable quality grade A and B while 6 (12.5%) and 11 (22.9%) were of grade C and D respectively. Nine embryos (18.8%) obtained were unfertilized indicating that infertility due to fertilization failure is higher in buffaloes (Table 8). The embryos obtained were transferred to recipients and pregnancy confirmed. The results suggest that single embryo recovery and embryo transfer (SOET) in buffaloes may prove to be a useful method for faster multiplication of superior females till more effective superovulatory regimes are available. This procedure also circumvents the non-availability of Buffalo Gonadotropin (FSH) which currently is a limitation to successful ET programme in buffaloes.

Follicular population

In buffaloes, the follicular system is not studied in detail as much as in cattle. Danell

Table 8 : Single ovulation and embryo recoveries in buffaloes.

Day of Cycle	Nos. attempted	Nos. recovered (%)	GRADE				
			UF	A	B	C	D
6	8	7	1	3	2	1	–
7	34	24	8	5	3	2	6
8	12	7	–	1	2	3	1
12 – 14	15	10	–	5	1	–	4
Total	69	48 (69.6)	9 (18.8)	14 (29.4)	8 (16..6)	6 (12.5)	11 (22.9)

(1987) studied the follicular system of cycling and noncycling Surti buffalo heifers for the number of primordial follicles and he found that the average number of primordial follicles were not significantly different ($P < 0.05$). The average numbers of follicles > 2.00 mm in cycling and non-cycling animals were 16.8 and 23.6 respectively which did not differ significantly. There were on an average twice as many atretic follicles as normal ones (31.7 and 14.6 respectively) in cycling animals. The average atresia frequency for all animals was 70.6 per cent. In a study on surface follicles, Mittal and Madan (1992) recovered on an average 4.3 follicles of < 2 mm, 1.0 > 2-4 mm, 0.45 > 4-6 mm, 0.14 > 6.8 mm and 0.17 follicle of > 8 mm and in over 60% ovaries cropus lutea was embedded and distinctly non-discernible on palpation. Further they found that the smaller size of ovary and CL was the reason for lower progesterone production.

Fertilization, embryo developmental pattern and embryo transport : A study was conducted on superovulated Murrah buffaloes (*Bubalus bubalis*) to record the fertilisation rate and development pattern (Singla *et al* 1992). A total of 139 embryos were recovered out of which 38 (27.4%) were unfertilised, 25 (17.9%) degenerated and 76 (54.7%) of transferable quality. The results suggest that though majority of embryos (44.5%) reach morula stage on day-5, 35.3% embryos had reached the stage of early blastocyst or beyond by day 6. This, when compared to cattle, buffalo embryos have a tendency to grow faster in the reproductive tract. The 7th day data on the limited number of observations further supports the above trend when it was observed that 13.8% of the embryos had reached fully expanded blastocyst stage on day-6 and 14.3% had reached hatched blastocyst stage on day-7. The data is suggestive that late ovulation, unovulated follicles and developmental pattern among early buffalo embryos, possibly, contribute to embryo-uterus asynchrony, thus resulting in low fertilization and higher embryonic mortality.

Oocyte culture and *In vitro* fertilization

One method to obviate the difficulties in embryo production responses among buffaloes is to utilize the *in vitro* culture and maturation techniques. The initial attempts with buffaloes (Mujumdar *et al* 1988, Singh *et al* 1988) have resulted in the production of up to 4 cell embryos. It was presumed that there exists a block in the development process. However, the work carried out at Embryo Biotechnology Centre of National Dairy Research Institute has successfully demonstrated the production of IVF embryos in buffaloes. In a series of trials oocytes were harvested from ovaries obtained from slaughter house giving a recovery of 1.03 oocyte per ovary compared to about 8 among bovines of exotic breeds. When subjected to *in vitro* maturation (Madan *et al* 1991, Madan *et al* 1992) 88% of the cumulus oocytes reached the mataphase II stage (Table 9). Using a oviduct epithelial co-culture system, sixty eight percent (Table 10) of the oocytes which were subjected to *in vitro* fertilization reached to more than 2 cell stage of deevelopment with 23% reaching the morula blastocyst stage. Attempts are in progress to use the transvaginal ultra sound guided puncture of ovarian stroma for aspiration of ovarian follicular ova directly (Kruip *et al* 1991) which shall help in knowing the pedigree and genetic merit of the oocytes material used in IVF work.

Table 9.: ***In vitro*** **maturation (IVM) of buffalo oocytes**

Medium	No. of oocytes stained	GV stage (%)	Met I (%)	Ana I (%)	Telo I (%)	Met II Matured stage (%)
TCM-199 with 10% Estrus Serum	184	17 (9.23)	23 (12.50)	3 (1.63)	3 (1.63)	147 (77.89)

Table 10 : ***In vitro*** **fertilization (IVF) of IVM buffalo oocytes.**

No. of Trials	No. of oocytes inseminated	Fertilization and No. of oocytes at	
		Pronuclei	> 2 cell
8	238	23 (9.66%)	163 (68.48%)

Conclusion

Observations on buffalo sufficiently justify that the physiology of buffalo particularly with respect to reproduction is different and should not be evaluated in poor light when compared with cattle. Several characteristic features have been observed in terms of superovulatory responses and reasons for limited embryo production identified. While considerable improvement in these responses has been achieved, further critical studies particularly in the area of folliculogeneseis, oocytes production *in vitro* fertilization, immunology of uterofetal interaction and fetal development need to be carried out to optimize productivity from this species.

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Effect Of Repeated Superovulation And Flushing On Embryo Recovery In Crossbred Cows

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ABSTRACT

To determine the effect of repeated superovulation and flushing in cross bred cows, 7 donors were repeatedly superovulated five times with an interval of 90-120 days between two superovulations. The mean number of ovulations recorded were 8.85±3.43, 7.28±2.92, 5.10±3.4, 3.50±2.30 and 2.75±2.21 in five attempts, respectively. The mean number of embryo recovery was 5.85±3.89, 5.0±2.2, 3.1±2.96, 2.5±2.6 and 2.5±2.6. The mean number of transferable embryo was 2.42±2.93, 4.42±2.50, 2.1±2.5, 2.0±2.5, 2.0±2.7 and 1.75±2.21. Repeated superovulatory treatments had a tendency towards

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decreasing number of CL, embryo recovery, and transferable embryos per cow.

* * *

Successful application of embryo transfer depends upon the superovulatory response on repeated treatment. Data on repeated superovulation in *Bos taurus* cows have shown that the number of ovulations decreased after repeated superovulation (Saumande and Chupin, 1977). In another study it was found that embryo production increases over three repeated superovulations and then declined for next four collections (Nelson *et al.*, 1979).

It was also observed that fertilization rate and embryo numbers decreases after 10 repeated superovulation on data analysis of nearly 1000 Holstein cows and heifers (Donaldsen and Perry, 1983). However, little is known about the effect of repeated superovulation in crossbred cows. Therefore, the aim of this study is to determine the effect of repeated superovulations in crossbred cattle.

Materials and Methods

Seven mature parous Holstein x Sahiwal (H x S) crossbred cows were used for five repeated superovulations. They were between 2nd and 4th lactation and provided adequate nutrition.

Each animal with a palpable corpus luteum (CL) on the ovary, received two doses of cloprostenol 500 µg 11 days (d) apart for the estrus synchronization.

Superovulation was induced with FSH-P in a four day decreasing dose schedule as per Totey *et al* (1988). All the donors were inseminated 0, 12 and 24 h. after estrus detection with freshly ejaculated semen. Interval between two superovulatory treatments ranged from 90 to 120 days. Before commencing next superovulation, donor females were observed for atleast two normal estrous cycles. Donors were also examined for evidence of possible reproductive problems.

Embryos were collected 7 d. after the last insemination by non-surgical technique using 18 french gauge foley's catheter (Urological Division, Bard Inc, Murray Hill, N.J. 07974) in Dulbecco's phosphate buffer saline (DPBS) containing 1% heat inactivated fetal calf serum, 100 IU sodium penicillin G and 100 ug streptomycin sulphate per ml. Embryos were collected by running the flushing medium through plastic embryo filter (Emcon Immunosystem Inc. ME 04043). A total of 1.0 to 1.5 litre of flushing medium was used per donor. The filter containing about 50 ml of flushing medium was brought to the laboratory. The sides and membrane of the filter were rinsed with fresh flushing medium. The flushed medium was examined under zoom stereo microscope. Located embryos were transferred to a petri dish containing the holding medium. Embryos were evaluated and graded morphologically at 70 times magnification and the data analyzed by one way analysis of variance.

Results and Discussion

It was observed that the number of flushes affected total ovulations, total number of embryos and number of transferable embryos. (Table 1). Mean number of total ovulations were 8.85±3.43, 7.28±2.92, 5.1±3.4, 3.5±2.3 and 2.75±2.21 in I, II, III, IV and Vth flush respectively. No other data are currently available relating to similar trend in cross bred cattle. However, in *Bos taurus* cattle the number of ovulations decrease after the second and fourth flush (Saumande & Chupin, 1977). Similarly in *Bos indicus* cattle total ovulations decreased from 9.4 at the first to 2.9 at fifth

flush (Bastidas and Randel, 1987). Monniaux *et al* (1983) suggested that the variations in the superovulatory response is due to differences in the follicular populations of ovaries, both in respect of number and stage of development. Long term effect of gonadotrophin on ovarian function has not been evaluated. However, there are possibilities of the production of antibodies against gonadotrophins (Greve, 1982)

The number of total embryos recovered were also affected with the subsequent flushes. Mean number of total embryos was high (5.85±3.89) for the first flush and low (2.5±2.6) at the fifth flush. There was a regular decrease of total number of embryos on repeated superovulations : 5.85±3.89, 5.0±2.2, 3.1±2.96, 2.5±2.6 and 2.5±2.6 respectively in I, II, III, IV and Vth attempt. Embryo production at the first collection was higher than that from cows superovulated three or more times. Donaldson and Perry (1983) reported that increase in the dose of gonadotrophin did not stop the decline in embryo production. Bastidas and Randel (1987) also reported that repeated superovulations decreases the embryo recovery ranging from 7.0 embryos per donor in the first flush to 3.0 embryos per donor in the sixth flush.

The mean number of transferable embryos also decreased with subsequent flushes. However, it was observed that the mean number of transferable embryos increased in second attempt than first (4.42±2.50 Vs 2.42±2.93). Viable embryos in the Vth attempt was as low as 1.75±2.21. Bastidas and Randel (1987) reported that the number of blastocysts and morulas recovered per donor cows were affected by repeated superovulation and flushing. The number of blastocyst recovered decreases rapidly after the fourth flush. Greve (1982) also found that number of ovulations was inversely related to the number of PMSG treatments. Similar trend was observed in terms of total number of eggs and viable embryos.

It is therefore concluded that repeated superovulations in cross bred cows has a detrimental effect on both production and quality of embryos recovered per flush.

Table 1. : **Superovulatory response in lactating crossbred cows repeatedly treated with FSH-P**

Attempts	Mean no of CL ± SD	Mean no of ova ± SD	Mean no of transferable embryo ± SD
I	8.85 ± 3.43	5.85 ± 3.89	2.42 ± 2.93
II	7.28 ± 2.92	5.00 ± 2.20	4.42 ± 2.50
III	5.10 ± 3.40	3.10 ± 2.96	2.10 ± 2.50
IV	3.50 ± 2.30	2.50 ± 2.60	2.00 ± 2.70
V	2.75 ± 2.21	2.50 ± 2.60	1.75 ± 2.21

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Comparative Efficacy Of Different Gonadotropin Preparations On Superovulation And Embryo Recovery In Jersey x Kankrej (J x K) Crossbred Cows

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ABSTRACT

Six J x K crossbred cows were repeatedly used 17 times for superovulatory response and embryo recovery rate, using four gonadotropin preparations. Average number of palpable corpora lutea and anovulatory follicles on day 7 post-breeding (estrus = Day O) for 3 different groups viz., Folltropin (Group-I), FSH (Group-II), and Folligon or PMSG (Group-III) treated cows were 8.80±2.82 and 3.60±1.44 (n=5), 6.57±0.53 and 3.43±0.68 (n=7) and 6.60±1.60 and 2.80±0.97 (n=5) respectively. Average number of embryos recovered per flush were 3.33±3.3, 3.25±0.63 and 1.20±0.49 in group I, II and III, respectively.

* * *

The success of superovulation is significantly influenced by factors such as species, breed, stage of estrous cycle, type and doses of gonadotropins (superovulatory agents) used and their purity (Donaldson and Perry, 1983); Saumande and Chupin, 1986; Savage *et al.*, 1987 and Bono *et al.*, 1991). Studies on superovulation using imported gonadotropins have been reported in India (Madan, 1990; Mishra *et al.*, 1992 a) in crossbred, zebu and exotic cows. However, there is lack of information on superovulatory response of gonadotropin preaparations produced within India (FSH and PMSG) and their comparative efficacy with imported preparations of gonadotropins (Folltropin) in crossbred cows. Present study is therefore undertaken.

Materials and Methods

Six pluriparous Jersey x Kankrej (J x K) crossbred cows 5 to 6 years of age with an average body weight of 325.12±6.13 kgs.kept under standard feeding and managerial practices at the RBRU farm were subjected to repeated superovulations (SOV) and embryo recovery. These cows were utilized alternatively for SOV, 17 times using four gonadotropin preparations : Folltropin (Vetrepharm Inc., Canada); FSH(Indian Immunologicals Ltd.(IIL),Hyderabad; PMSG(IIL) and Folligon (Intervet, Holland) after a rest of at least two estrous cycles postflushing (50 to 60 days). The induction of SOV in the donor animals (Group I & II) was started on day 11 or 12 of natural estrus, by injecting (IM) follicle stimulating hormone (FSH) twice daily for 4 days in descending dose schedule. The preparations and their total dose used were : folltropin (35 mg) or FSH (44 mg). A total dose of 35 mg prostaglandin F_2alpha (Lutalyse) was injected at 84 and 96 hours of initiation of SOV treatment. Group-III donors were treated with single IM injection of folligon or PMSG (2200-2500IU) on day 9 to 12 of estrous cycle. Prostaglandin was injected 48 hour after PMSG injection to induce luteolysis. Superovulated animals were inseminated 1 to 3 times after induced estrus. Rectal palpations were carried out during pre-selection, estrus and on day of flushing to record follicles and corpora lutea formation. Embryos were collected by standard non-surgical technique on day 7 post-breeding (estrus = day O) in Dulbecco's phosphate buffer saline medium (sigma USA/Himedia, Bombay) fortified with 0.1% bovine serum albumin and antibiotics using 2 way Foley or Rusch Catheter, 18 French Gauge (Medizintechnik, Germany). Embryos were evaluated under stereomicroscope at magnification of 40x. Statistical analysis of the data was done as per Snedecor and Cochran (1971).

Results and Discussion

In this study, mean number of corpora lutea were higher in folltropin group (8.8±2.82) compared to FSH (IIL) and PMSG treated groups (6.57±0.53 and 6.60±1.60). Similar trend was observed for total ovarian response in terms of formation of corpora lutea as well as anovulatory follicles (Table 1). Mean number of good quality embryo recovered was 3.33±3.30, 3.25±0.63 and 1.20±0.49 in groups I,IIand III, respectively.

From the present study, it seems that folltropin is better SOV agent as compared to FSH(IIL) and PMSG. The study further suggests that average number of embryos recovered per flush were higher in folltropin and FSH(IIL) groups compared to PMSG treated group. Moreover, effect of folligon and PMSG(IIL) to induce SOV was quite similar. However, large data is needed to substantiate these findings.

Madan (1990) reported 4.63 ovulation rate and 4.25 embryo recovery in Karan Fries cows using folltropin. Mishra *et al.* (1992 a,b) and Pawshe *et al*,(1992) reported better superovulatory response in Holstein and Jersey crossbred cows compared to present findings.

Monniaux *et al.* (1983) and Bono *et al.* (1991) obtained significantly lower ovulation rate by PMSG compared to FSH-LH(Sigma) 50mg preparations. Present findings are comparable with those of Brown *et al.* (1990), where overall mean ovulation rate and number of good quality embryos recorded per flush were 7.4 and 1.3 respectively.

Eldsen *et al.* (1978) found no difference between PMSG and FSH treatment response. Most of the the workers stated that PMSG

which has been widely used and is economical has shown to be less effective in controlling superovulation (Saumande, 1980; Lauria *et al.*, 1982) resulting in aberration in follicular development (Dieleman and Kruip, 1980 and Hyttel *et al.*, 1986). This negative effect may be due to the long half life of this particular gonadotropin (Lauria et *al.*, 1982).

Therefore, it can be concluded that folltropin is better superovulatory agent, compared to FSH (IIL) and PMSG in J x K crossbred cows. However, further studies are required to substantiate these findings.

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Table 1 : **Superovulatory response of different gonadotropin preparations in Jersey x Kankrej crossbred cows**

Groups	Superovulatory agents	No.of donors treated	Mean ovulations	Mean anovulatory follicles	Total ovarian response	Mean good quality embryos
I	Folltropin (FSH)	5	8.80 ± 2.82	3.60 ± 1.44	12.40 ± 2.80	3.33 ± 3.30 (3)
II	FSH (IIL)	7	6.57 ± 0.53	3.43 ± 0.68	10.00 ± 0.90	3.25 ± 0.63 (4)
III	PMSG (Folligon or PMSG, IIL)	5	6.60 ± 1.60	2.80 ± 0.97	9.40 ± 2.24	1..20 ± 0.49 (4)

Number in parentheses indicates number of donors flushed

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Superovulatory Response With FSH-P And Ovogen In Bovines

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ABSTRACT

The superovulatory response with 32 mg FSH -P was better ($p<0.05$) than 1.3 u Ovogen. In all, only 36% of the treated animals yielded embryos. In 32 mg FSH-P administered group, 55.55% cows yielded embryos, whereas, in Ovogen group only 28.57% cows yielded embryos. In buffaloes there was no response with 40 mg FSH-P. The mean number of palpable corpora lutea and anovulatory follicles in superovulated cows were 9.77±1.93 and 1.44±0.26, respectively. The number of corpora lutea and follicles were more in right ovary than the left, however, the difference was non-significant. In most cases the number of corpora lutea palpated in the ovaries did not corroborate with the number of embryos flushed. Of the total embryos flushed, 23.26% were of good, 11.63% average and rest 65.11% were of poor quality. The occurrence of degenerated embryos or oocytes increased with increasing number of embryos received from a donor. A good morula transferred non-surgically, resulted in successful conception. However, a morula and a blastocyst of good quality, when transferred surgically, the recepients repeated after 32 and 42 days, respectively.

* * *

Very little is known about the factors which influence the rate of recovery of eggs in superovulated animals (Curtis, 1990). In the present study superovulatory response was elucidated after administering FSH-P and Ovogen to the different groups of crossbred cows and buffaloes. Good quality embryos were transferred non-surgically and surgically.

Materials and Methods

Total 25 donor (23 crossbred cows and 2 Murrah buffaloes) and 3 recipient(2 crossbred cows for surgical and 1 for non-surgical transfer) animals were administered the superovulatory regimen.

Different gonadotrophin doses were given (Table 1) : Group I (9 cows received 32 mg FSH-P), Gr.II (7 cows received 1.3 u Ovogen),

* Part of the training course on Embryo Transfer Technology held at the National Biotechnology Centre, Indian Veterinary Research Institute, Izatnagar, March, 1991.

Table 1 : Doses of gonadotrophins administered for superovulation in experimental groups.

Donor Animals	Group and dose	Days of oestrous cycle and time of administration							
		11th Day		12th Day		13th Day		14th Day	
		A. M.	P. M.	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.
Cross-bred cows	Group-I FSH-P*32mg	6	6	4	4	4	4	2	2
Cross-bred cows	Group-II Ovogen** 1.3 U (13 ml)	3	3	2	2	1	1	½	½
Cross-bred cows	Group-III Ovogen 1.5 U (15 ml)	3	3	2	2	1½	1½	1	1
Buffalo	Group-IV FSH-P 40 mg	7	7	6	6	4	4	3	3

* Follicle Stimulating Hormone – Pituitary for injection. (Schering Corporation. U.S.A.)

** Follicle Stimulating Hormone – Ovine Pituitary Extract. [Immuno Chemical Products Limited Auckland. (N.Z.)]

Table 2 : Mean superovulatory response of the embryo yielding donors.

Group	No.of animals receiving superovulatory treatment	Animals which yielded embryos (%)	Mean No.of corporalutea in embryo yielding donors	Mean No.of follicles in embryo yielding donors	Mean No.of embryos		
					Good	Average	Poor
I	9	55.55	10.80	1.40	1.20	1.00	5.40
II	7	28.57	3.00	0.50	1.00	0.00	0.50
III	7	28.57	14.00	2.50	1.00	0.00	0.00
IV	2*	00.00	1.00	0.00	0.00	0.00	0.00
Overall	25	36.00	9.77 ± 1.93	1.44 ± 0.26	1.11 ± 0.17	0.55 ± 0.22	3.11 ± 1.54
Quality of embryos (%)	-	-	-	-	23.26	11.63	65.11

* These animals were excluded for calculating overall means.

Gr.III (7 cows received 1.5 u Ovogen) and Gr.IV (2 buffaloes received 40 mg FSH-P). FSH treatment (IM) was started from day-11 in tapering doses at 12 hourly interval upto 4 days. Dinofertin, 25 mg was administered in the morning of day-14. Within 48 hours of Dinofertin injection, whenever the animal expressed standing heat, two inseminations were done at 12 hourly interval. Embryo recovery was done between day 7 and 8 by 3-way Foley's catheter. Dulbecco's phosphate buffered saline with foetal calf serum was used as the medium for flushing.

A good quality fresh morula from donor No. 1040 D was transferred non-surgically to the cow No. 038 at almost same synchrony. For surgical transfer, a good quality morula received from the donor No.1040 D was deposited in the recipient No. 087. Similarly, a good blastoyst received from the donor No. 786 F was deposited in the recipient No. 2177. Both the recipients had characteristic C.L. of day-7 on the left ovary, hence one embryo was deposited in the left uterine horn in each case surgically.

Results

The superovulatory response with 32 mg FSH-P proved significantly better ($P < 0.05$) than Ovogen at 1.34 or 1.54 u dose rates. The superovulatory response showed great variation between animals-within group (Table-2). In Group IV, where 40 mg FSH-P was injected to the buffaloes there was no response. The number of palpable corpora lutea/anovulatory follicles were more in right (51/15) compared to the left (44/13) ovaries. However, the difference was non-significant. When number of embryos harvested were more from a donor, the occurrence of degenerated embryos or oocytes also increased. In most cases the number of palpable corpora lutea in the ovaries did not corroborate with the number of embryos flushed.

In all 36% of the treated animals yielded embryos. In Group I, embryos could be flushed from 55.55% cows followed by 28.57% in Group II and III. The mean numbers of corpora lutea and anovulatory follicles in the superovulated cows were 9.77 ± 1.93 and 1.44 ± 0.26, respectively. Of the total 43 embryos flushed 23.26% were of good, 11.63% average and rest 65.11% of poor quality.

The morula of donor No. 322 B transferred non-surgically to the recipient No. 038 conceived successfully. However, a morula and blastocyst when transferred surgically, the recipients repeated after 32 and 42 days of transfer, respectively.

Disucussion

As observed in the present study, other workers have also reported tremendous variation in superovulatory response in cattle (Bhattacharya *et al.*, 1987) and buffaloes (Boothipakhsa, 1988). Pant (1987) described that several gonadal peptides that bind gonadotrophins to their receptor proteins may contribute to reported variability in various superovulatory regimens. Chantaraprateep *et al* ., (1987) also obtained poor superovulatory response in buffaloes using FSH-P.

Hafez (1989) stated that many of the ova flushed from superovulated animals are unfertilized and this is one of the greatest limitation in embryo transfer technology.

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A Record of Parthenogenic Oocyte Matured In Vitro In Buffalo (Bubalus bubalis)

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Development of embryo without intervention of sperm which is known as parthenogenesis, has been recorded as of rare occurrence in many invertebrate and in some vertebrate species (Hafez, 1987). Such a spontaneous formation of embryo devoid of fertilization has been attributed to inherent tendency of proliferation and differentiation of female germ plasm. It is interesting to note that it has been possible to initiate parthenogenesis experimentally by briefly exposing oocytes to 100 IU/ml hyaluronidase in culture medium in mouse (O' Neill and Kaufman, 1988) and by exposing briefly to 7-9% ethanol in mice (Han *et al.*, 1987).

In bovines it is worthwhile to record that oocytes cultured *in-vitro* for 24 hours and transferred to rabbit fallopian tube showed morphological alteration indicating 8% parthenogenetic activity after 24 hours (Trouson *et al.*, 1977). In similar work 15-20% of success in getting such oocytes was obtained by Xu *et al.*, (1986) and Wall and Hawk, (1988). A direct electric current with pulse of 1KV for 25/u sec x 2 given to oocytes was observed to give 46% success getting parthenogenetic

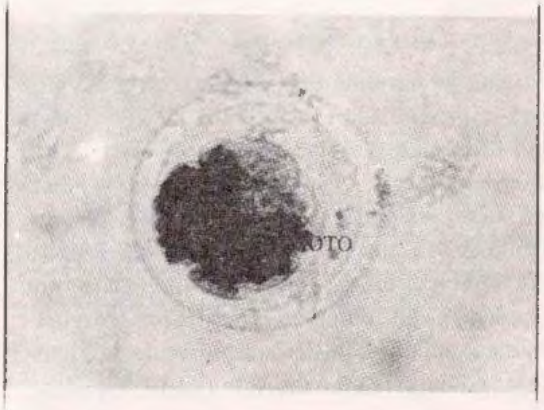

Fig. 1 : Parthenogenetic oocyte of buffalo showing eight cell stage (150-X)

change (Kono *et al.*, 1989). Although this resulted into development of an oocyte to blastocyst stage, it was never possible to have successful term pregnancy in any mammalian species with such oocytes (Daniel, 1978).

In our study carried out on morphological maturation of buffalo oocytes *in-vitro* at 24, 48 and 72 hours, 140 oocytes were aspirated from follicles of the size > 3mm from 68 pairs of ovaries of slaughtered buffaloes. Of these, 126 oocytes were placed in M-199 medium (Hi-media, Pvt. Lab. Ltd., Bombay) supplemented with 25 mM HEPES buffer, 20% heat inactivated (56°C for 30 minutes) buffalo estrous serum (BES) and 50 ug gentamycin per ml with pH 7.4 and incubated at 38°C in 100% air (humidified) atmosphere for 24, 48 and 72 hours. During detailed observations on 116 oocytes after 24, 48 and 72 hours, a solitary oocyte (0.86%) was found to show parthenogenetic change with eight cell stage of embryo within the zona pellucida (Fig. 1). The measurements of this oocyte did not differ from the normal oocyte.

On scanning the available literature, the present record of spontaneous parthenogenic activity (0.86%), appears to be the first of its kind reported in buffalo species.

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A Study On The Effect Of Holding Straws At Various Termperatures On Freezing Of Bull Semen.

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ABSTRACT

Bull semen diluted in egg yolk Tris glycerol diluent packed in medium straws and kept directly in water baths at 5°C to 30°C for 15 minutes did not produce any adverse effect on pre-freeze motility. Possibly the yolk, glycerol and packing material (Polyvinyl chloride) provided adequate protection against cold shock. It is concluded that normal cooling of straws for freezing could also be started

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directly at lower temnperatures (10°C or 15°C) than at 30°C. However, post-thaw motility was highest for semen treated at 5°C, though there was no significant difference between 5°C, 10°C or 15°C. This key modification in semen processing technology was subsequently used in the development of faster test-freezing methods.

* * *

Since first report of bull semen freezing process by Polge (1952), the method involved two basic steps : (i) slow cooling of the diluted semen to +5°C (±1°C) and in most cases followed by equilibration at +5°C (±1°C) and (ii) Deep-freezing of the equilibrated/cooled semen (+5°C) in dry ice/liquid nitrogen. In all the available reports, actual deep freezing was done on cooling diluted semen (whether equilibrated or not) to +5°C (±1°C). But is it essential to start cooling diluted semen from 30/37°C and then lower it down 5°C (±1°C)? Is it not possible to place the diluted semen at 30/37°C with sufficient cryoprotectant (Egg yolk), directly to lower temperatures 'x' without cooling it specifically to +5°C (±1°C)?

The present study was conducted to find out the minimum initial cooling temperature which can be resisted by the spermatozoa after dilution of semen with tris-glycerol-eggyolk dilutor so that the subsequent cooling to 5°C could be started directly from such minimum initial temperature instead of from 30/37°C (as is being generally done) to reduce the total cooling time to +5°C. These modifications in processing technology may reduce the operational period and make the frozen semen techniques more efficient. A beginning was made to achieve some of these objectives with the ultimate aim of developing a rapid test freezing method which could predict the freezability of semen within 45 minutes after collection (Mathur, 1989).

Material and Methods

The study involved a total of 15 first ejaculates (3 per bull) from five Holstein-Friesian bulls. Ejaculates with less than 40% initial motility were rejected.

The semen immediately after collection and evaluation was diluted with tris-eggyolk-glycerol dilutor (containing 20% eggyolk and 7% glycerol) at 30°C to have 50 million sperm/ml. This diluted semen was filled in 0.5 ml French straws (20 straws per sample) and sealed with polyvinyl powder. Immediately after filling, the straws were transferred to five water baths at different temperatures representing five treatments. 4 straws per treatment were taken. The treatments tried were :

1. *Treatment 1 (T-1) :* Direct placement and maintenance of straws at 30°C water bath for 15 minutes.

2. *Treatment 2 (T-2) :* Direct placement and maintenance of straws at 20°C water bath for 15 minutes.

3. *Treatment 3 (T-3) :* Direct placement and maintenance of straws at 15°C water bath for 15 minutes.

4. *Treatment 4 (T-4) :* Direct placement and maintenance of straws at 10°C water bath for 15 minutes.

5. *Treatment 5 (T-5) :* Direct placement and maintenance of straws at 5°C for 15 minutes.

Ordinary rectangular plastic bread boxes (22 cm x 11 cm) each having about 400 ml water at respective temperatures were used as water baths. The water bath termperature was maintained by addition if required, of ice or warm water. After 15 minutes, the straws were taken out from the water baths. One straw from each box was used for assessing prefreeze

Table 1 : **Mean (± SE) value of different treatments for prefreeze and post-thaw motility : Experiment I.**

Evaluation Parameter*	Bull No.	T 1	T 2	T 3	T 4	T 5
1. Prefreeze Motility %	B1	46.66 ± 1.66	50.00 ± 0.00	43.33 ± 9.28	41.66 ± 7.26	46.66 ± 3.33
	B2	53.33 ± 8.81	53.33 ± 6.66	50.00 ± 8.66	46.66 ± 6.00	50.00 ± 5.77
	B3	40.00 ± 7.63	50.00 ± 0.00	46.66 ± 4.41	50.00 ± 2.88	41.66 ± 10.92
	B4	56.66 ± 3.33	60.00 ± 2.88	58.33 ± 1.66	58.33 ± 1.66	58.33 ± 1.66
	B5	31.66 ±15.89	38.33 ±11.66	33.33 ±13.01	36.66 ±14.53	30.00 ±14.45
	Over all	45.66 ± 4.16	50.33 ± 2.98	46.33 ± 3.85	46.66 ± 3.57	45.33 ± 4.12
		(42.05 ± 2.7)	(45.11 ± 1.78)	(42.60 ± 2.40)	(42.81 ± 2.24)	(41.80 ± 2.71)
2. Post-thaw Motility %	B1	5.66 ± 0.66	11.66 ± 1.66	16.66 ± 1.66	18.33 ± 1.66	16.66 ± 4.41
	B2	6.66 ± 1.66	10.00 ± 1.00	13.33 ± 3.33	13.33 ± 3.33	20.00 ± 5.77
	B3	8.33 ± 1.66	18.33 ± 1.66	28.33 ± 7.26	26.66 ± 7.26	30.00 ± 2.88
	B4	15.00 ± 2.88	11.66 ± 4.41	13.33 ± 3.33	25.00 ± 0.00	16.66 ± 3.33
	B5	1.66 ± 1.66	6.66 ± 1.66	6.66 ± 1.66	6.66 ± 1.66	8.33 ± 1.66
Over all		a	c	abc	ab	a
		7.46 ± 1.35	11.66 ± 1.35	15.66 ± 2.43	18.00 ± 2.43	18.33 ± 2.37
		(14.39 ± 1.84)	(19.53 ± 1.21)	(22.56 ± 1.83)	(24.37 ± 1.87)	(24.68 ± 1.80)

Note : *(1) The mean values for Post-incubation and Post-ageing motility were less than 5% hence not reported.
(2) Figures in parentheses indicate overall mean after arcsin percentage transformation of the data.

motility, while the remainng three straws were frozen by the two step freezing method of Jondet *et al* (1980).

Thawing was done at 37°C for 30 seconds. After thawing, out of 3 straws, one was used for assessing post-thaw motility, second was kept in incubator at 37°C for one hour for assessing post-incubation motility and the third straw was kept in refrigerator at 5°C (in thawing water) for 24 hrs. for assessing post-ageing motility.

Results

The overall seminal characteristics were: initial motility 59.00±2.81%; sperm concentration 698.00±100.47 millions / ml; dead sperm 31.13±3.68% and abnormal sperm 9.60%. Out of the five bulls, one (No 701) was having consistently poor semen quality (mean initial motility 45%, dead sperm 45-66%) due to which the over-all mean for initial motility was low and that of percent dead sperm was high.

Table 2 : **Analysis of Variance for the effect of treatments on Prefeeze and Post-thaw Motility**

Sources of Variation	D.F.	Prefreeze M.S.S.	Motility 'F' Value	Post-thaw M.S.S.	Motility 'F' Value
1. Between treatments	4	25.99	0.326 NS	274.14	13.01*
2. Between bulls	4	477.26	6.004**	419.35	19.91**
3. Interaction (Bull X treatment)	16	10.10	0.127 (NS)	26.28	1.24 (NS)
4. Error	50	79.48	-	21.05	-

NS = Non-Significant. * = Significant at 5% level. ** = Significant at 1% level.

Analysis of variance of the data for prefreeze and post-thaw motility was done by two way analysis. The values of post-incubation and post-ageing motility were less than 5%, so they have not been reported. The significantly different means were compared by critical difference among treatments.

The mean values for prefreeze motility for the five treatments (T-1 to T-5) were: 45.66 ±4.16; 50.33±2.98; 46.33±3.85; 46.33±3.57 and 45.33±4.12% respectively (Table-1). There was non-significant difference between treatments but with a highly signficant (P<0.01) difference between bull.

The mean post-thaw motility values (T-1 to T-5) were: 7.46±1.35; 11.66±1.35; 15.66± 2.43; 18.00±2.43 and 18.33±2.37% respectively. There was a highly significant difference (P<0.01) between treatments and between bulls (Table-2). Among treatments, the grading for post-thaw motility values was in the following sequence T-5, T-4, T-3 T-2 & T-1 but there was no significant difference between T-3, T-4 & T-5 and between T-2 & T-3.

Discussion

The non-significant difference (T-1 to T-5) indicated that semen diluted at room temp. immediately after collection could be placed directly to a temp. of 30°, 20°, 15°, 10°C and even at 5°C without any significant adverse effect on its prefreeze motility. It further showed that bull spermatozoa in diluted semen packed in straws were not cold-shocked even by their direct placement to temp. ranging from 30°C to 5°C for 15 minutes, supporting the earlier observations of Easley (1942), Blackshaw (1954) and Blackshaw & Salisbury (1957). It suggested that cooling of diluted semen to 5°C for the normal freezing process could be started from lower temperatures than is the practice hitherto by the direct placement of filled straws to such initial lower temperatures.

The aim of freezing semen after maintaining it for 15 minutes at temperature of 30°, 20°, 15°, 10° and 5°C for 15 minutes (T-1 to T-5) was to note the relative cryoresistance developed by spermatozoa at these varying temps. Post-thaw revival indicated that the cryoresistance was highest for T-5(5°C) and lowest for T-1(30°C) with non-significant difference between T-3 (15°C). T-4(10°C) and T-5(5°C) values. The post-thaw motility values regularly increased as the temperature (30°C to 5°C) decreased. It indicated that best cryoresistance was achieved by incubation of spermatozoa at 5°C before freezing, which supported the existing practice of cooling and equilibrating spermatozoa to 5°C before freezing. Similar previous reports could not be traced in literature. The key observation from this study was utilized by cooling diluted semen at lower initial temperature of 20°C (T-2) 10°C (T-4) & 5°C(T-5) for developing faster Test freezing methods (Mathur, 1989).

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Semen Freezability In Relation To Single Or Multiple Exotic Genetic Component In Crossbred Bulls

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ABSTRACT

Semen freezability in 30 crossbred (CB) bulls with one (J) or multiple (J, HF or BS) exotic breed were studied. Of the 19 CB bulls with only Jersey exotic component (Group A), semen of 8 bulls was freezable (42.11%). In other group (B) of 11 crossbred bulls with more than one exotic component, semen of 2 bulls was freezable (18.18%).

Co-efficient of variation (C.V.) of post thaw motility in group A was 32.34% indicating lesser variation, whereas in Group B, it was 50.52% indicating poor freezability and greater variation. It is apparent that the freezability of Crossbred bulls semen is inversely proportional to their exotic genetic component.

* * *

Availability of Crossbred bulls semen is an enigma due to problems of its freezability. Present studies on freezability of crossbred bulls semen were undertaken in relation to the exotic genetic make up of these bulls.

Material and Methods

These studies were conducted on 30 selected crossbred(CB) bulls between 18 to 21 months of age, located at Frozen Semen Station, Nagpur over a period of 1½ years (1989-91). Group A crossbred bulls comprised of three 75 % Jersey and 16, 62.5 % Jersey crosses only. Group B bulls comprised of two 75% Jersey and 9,62.5% CB bulls with 12.5 to 25% levels of Jersey, Holstein, Friesian or Brown-Swiss breeds. The indigenous inheritance in these crossbred bulls varied between 12.5 to 37.5% of Gaolao, Hariyana, Gir and Khillar. All these crossbred bulls were in good health, free from diseases and maintained under identical managemental conditions.

Semen ejaculates were collected by A.V. technique twice a week. Semen samples having density DD and above, mass activity++

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and above and initial motility 70% and above were subjected to freezability. Tris egg yolk glycerol (6%) dilution was used. Antibiotics were added to the dilutor @ 1 mg/ml. Freezing was carried out in .25 ml straws (Landshut method) with equilibration time of 4 hours at 4°C. Horizontal vapour freezing technique was used as per Mathew *et al* (1974). Post thaw motility (PTM) was observed 30 minutes after freezing. Thawing was carried out in 37°C water for 15 seconds. Frozen Semen samples having minimum 40% motility and above were considered as freezable. The relevant data was statistically analysed as per Snedecor and Cochran (1967).

Results and Discussions

1. *Volume* : The mean semen volume of 3.56±0.16 ml was recorded in Group A Jersey crossbred bulls with C.V. 56.74%, whereas in group B bulls mean semen volume was 3.66±0.16 ml and C. V. 47.81% (Table 1).

Table 1 : Mean, Standard error and Coefficient of Variation of various semen characteristics of crossbred bulls with one or more exotic genetic components.

Sr. No.	Character		N	Mean	S.E. ±	C.V. (%)
1	Volume (ml)	A	168	3.56	0.16	56.74
		B	115	3.66	0.16	47.81
2	Mass activity (0-5)	A	149	1.77	0.08	54.80
		B	112	1.76	0.08	42.61
3	Initial Motility	A	147	35.50	2.17	73.44
		B	112	36.12	2.14	62.57
4	Post-thaw Motility(%)	A	40	40.13	2.05	32.34
		B	20	32.50	3.67	50.52

Note : A = One exotic breed component.
B = More than one exotic breed component

Lower values were recorded by Sagdeo *et al* (1990) in case of 75% Jersey crossbred bulls (2.23±0.18 ml). Bakshi (1980) also recorded slightly lower values (3.2 to 3.5 ml) in case of 75% HF crossbred bulls. Slightly higher values (3.73±0.24 ml) were recorded by Sagdeo *et al* (1990) in case of 62.5% Jersey Crossbred bulls. Sharma *el al* (1986) recorded higher values (4.6±0.4 ml) in 11 crossbred bulls (BS X HF X Gir). Raja and Rao (1983) also recorded higher values (3.73±0.24 ml) in 62.5% Jersey crossbred bulls.

2. *Mass Activity* : Mean mass activity recorded in Group A bulls was 1.77±0.08 with C.V. 54.80%, whereas in Group B it was 1.76±0.08 and C.V. 42.60%. Similar values (1.77±0.01) were recorded by Sagdeo *et al* (1990) in 75% HF crossbred bulls. Baburao and Rao (1990) and Sharma *et al* (1980) recorded higher values-3.48±0.17 and 3.80±0.1 respectively.

3. *Initial Motility* : Mean intial motility of 35.50± 2.15 with C.V.73.44% was recorded in Group A and 36.12±2.14, C.V.62.57% in Group B bulls. Sagdeo *et al* (1990) recorded lower values for initial motility (30.24±2.94) in case of 75 % HF crossbred bulls. However, Chauhan *et al* (1983) recorded higher mean values (65.77±3%) in 75% crossbred bulls. Mathew *et al* (1982) also recorded higher values in case of 75% Brown Swiss Crossbred bulls between age of 2-4 (63.98%) and 4-6 years. (67.5%). In both the groups under study, there was great variation in initial motility with C.V. 73.44% (Group A) and 62.87% for Group B.

4. *Post Thaw Motility (PTM)* : Mean PTM for Group A was 40.13±3.67% for Group B. There was less variation (C.V.32.34%) in Group A than in case of Group B (C.V. 50.52%). Sagdeo *et al* (1990) recorded lower PTM values (28.33±5.26%) in 75% Jersey crossbred bulls, but Mathew *et al* (1982) recorded higher PTM values in 62.5% Jersey

crossbred bulls (44.79%) and higher values (49.39%) in 75% Brown-Swiss crossbred bulls.

In Group A, 7 out of 19 crossbred bulls were rejected as initial motility was not upto the mark and 4 bulls were rejected as PTM was below 40%. In all, 11 crossbred bulls (57.89%) were rejected and 8 crossbred bulls (42.10%) were found to be freezable. Out of 168 semen samples, only 40 samples (23.80%) in Group A were put to freezability test.

In group B out of 11 crossbred bulls, 4 were rejected as initial motility was below 70% and 5 bulls were rejected due to poor PTM. In all, 9 crossbred bulls (81.81%) were rejected and only 2 crossbred bulls semen was found to be freezable (18.18%). Out of 115 semen samples in this Group(B), only 20 samples (17.39%) were tested for freezability.

Analysis of variance for volume, mass activity, initial motility and PTM showed no significant difference among these two groups indicating that though both the groups responded to freezability trials, a large variation in the percentage of freezable bulls in each group was found.

However, while selecting crossbred bulls for freezability test consideration has to be given to the exotic breed component included in such crossbred bulls. With increase in the number of exotic breed components, lesser number of such crossbred bulls were found to be freezable. Climatic effect may be an additive factor for low freezability which needs further investigation.

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The Use Of Liquid Nitrogen Vapours For Cooling Of Semen Straws To 5°C

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ABSTRACT

An attempt was made to cool straws to 5°C (pre-freeze) in liquid nitrogen vapour (LNV) itself eliminating the use of refrigerators. This is necessitated because of frequent cuts or breakdown in electric supply in most of the developing countries located in tropics causing serious set-back to frozen semen technology.

The use of simple experimental approaches by trying the cooling of straws in plastic thermowares containing fixed quantities of LN_2 and vapourised at fixed heights either at a wire mesh or wrapped in cotton plug have been successfully tried and results of freezing have been compared with the cooling of semen achieved in freezing chamber of the refrige- rator. Results of freezability of cattle semen were better in treatments involving LN_2 vapour.

* * *

Cooling of semen filled straws from about 30°C to 5°C constitute an important step in cattle semen freezing technology. The optimum cooling time (to 5°C) is quite variable depending on many factors. The method of cooling straws from 30/35°C to 5°C also differ. The most common method of cooling is to keep the diluted semen in water jacket in refrigera- tor at 5°C. Another method reported to be useful in tropics consists in slow cooling in freezing chamber of the refrigerator (Sahni and Mohan, 1988a, b). Gilbert and Almquist (1978) placed filled straws in card-board trays inside a covered card-board box kept at 5°C in refrigerator. The use of refrigerator is common in all these methods.

In the present experiment, an attempt was made to cool the semen filled straw to 5°C in liquid nitrogen vapour (LNV) itself and to compare it with freezing chamber method of cooling. The objective was to explore the possibilities of using LNV for both cooling to 5°C and subsequent deep-freezing, avoiding use of refrigerator which sometimes becomes a limitation due to erratic power supply in many parts of the developing countries especially for the freezing stations located in the semi-urban areas. This study was part of the project undertaken (Mathur, 1989) to develop some rapid test freezing methods for predicting the freezebility of a semen sample within 45 minutes (mts) after collection. No earlier report of cooling straws to 5°C in LNV could be traced out in the literature.

Materials and Methods

The study involved 15 first ejaculates from five Holstein Friesian (HF) bulls (3 ejaculates per bull). Ejaculates having less than 40% initial motility were rejected.

The semen immediately after collection and initial evaluation was diluted with Tris-egg-yolk-Glycerol dilutor (containing 7% glycerol and 20% eggyolk) at 30°C. Diluted semen was filled in 0.5 ml French straws. After

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sealing with polyvinyl powder the straws were subjected to the following cooling (to 5°C) treatments (four straws per treatment).

1. *Treatment 1 (T-1)* Involved direct placement of straws at 10°C in LNV followed by cooling to 5°C in 15 mts.

2. *Treatment 2 (T-2)* Involved direct placement of straws at 10°C followed by cooling to 5°C in 15 mts in freezing chamber of the refrigerator.

3. *Treatment 3 (T-3)* Involved direct placement of straws in 10°C water followed by their cooling to 5°C in 30mts in freezing chamber of the refrigerator.

4. *Treatment 4 (T-4)* Involved direct placement of straws, protected inside a cotton-plug in LNV at 5°C for 15mts,

5. *Treatment 5 (T-5)* Involved direct placement of straws protected inside a cotton-plug in LNV at 5°C for 30mts.

The direct placement of straws at 10°C or 5°C temperature was adopted from the observations of the previous two experiments under the project (Mathur, 1989).

Procedure

Treatment (T-1) : Cooling of the straws was done in a thermoware container (Milton five star ®) 32 cm in height and 15cm internal diameter (Fig.1) One double layered cardboard case was fitted inside the thermoware for protecting the wall of the container by the frequent updown movements of the wire-mesh plate (used to hold straws within thermoware) and to increase the workable height of the container.

Standardisation was done earlier by using varying amount of LN_2 and noting the temperature at various heights using wire thermocouple fitted to wiremesh plate having fixed number of straws filled with diluted semen of 30°C. Thus by using 300ml LN_2 the temperature at a specific height was 10°C which used to fall down to 5°C in 15minutes. This specific point was marked and shall be referred as 10°C temperature height. This point remained constant for the entire experiment except with occasional minor adjustments made for getting initial 10°C temperature required due to sharp change in surrounding temperature.

Immediately before use, the thermoware was primed by pouring about 20-50 ml of LN_2 in the container followed by gentle shaking of the container. Then 300ml of LN_2 was transferred to the container, the wire-mesh plate was fitted at the 10°C height mark and connected with wire thermocouple. As soon as the temperature became stationary at 10°C the four semen filled straws (maintained at 30°C after sealing) were spread horizontally on the plate. The lid was replaced on the thermoware. When the temperature reached to 5°C which took 15-20 minutes, the straws were taken out, evaluated and frozen.

Treatments 2 & 3 : Immediately after filling and sealing, 4 straws each were transferred to two separate plastic rectangular "Bread Boxes" (22cm x 11cm) which were having 225 ml (for T2) and 325 ml(for T3) water at 10°C. The boxes were then placed in the freezing chamber of the refrigerator and were removed after 15 minutes and 30 munutes respectively. Further evaluation and freezing process has been described later.

Treatments 4 & 5 : In these treatments 4 straws each were wrapped in separate non-absorbant cotton plugs of fixed specification (weight 12.75gm: size 19cm X 9cm). These cotton plugs were then directly placed at 5°C temperature height in another thermoware

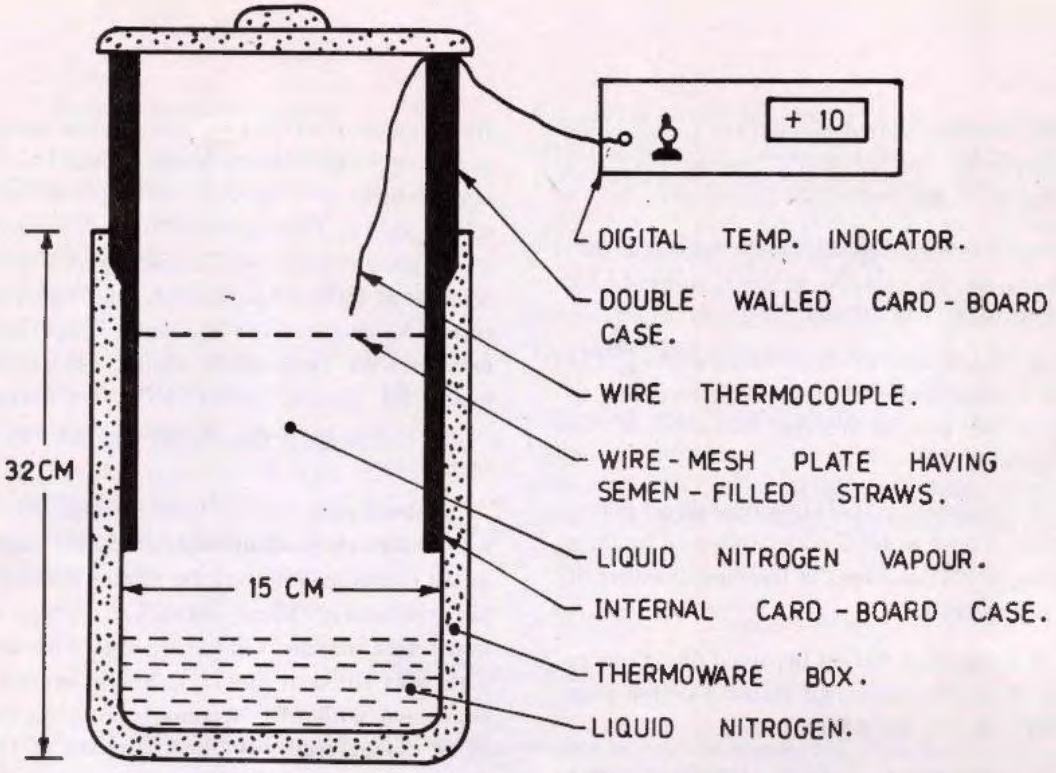

FIG. 1 - DIAGRAMATIC REPRESENTATION OF THE ASSEMBLY USED FOR COOLING SEMEN - STRAWS IN LIQUID NITROGEN VAPOUR.

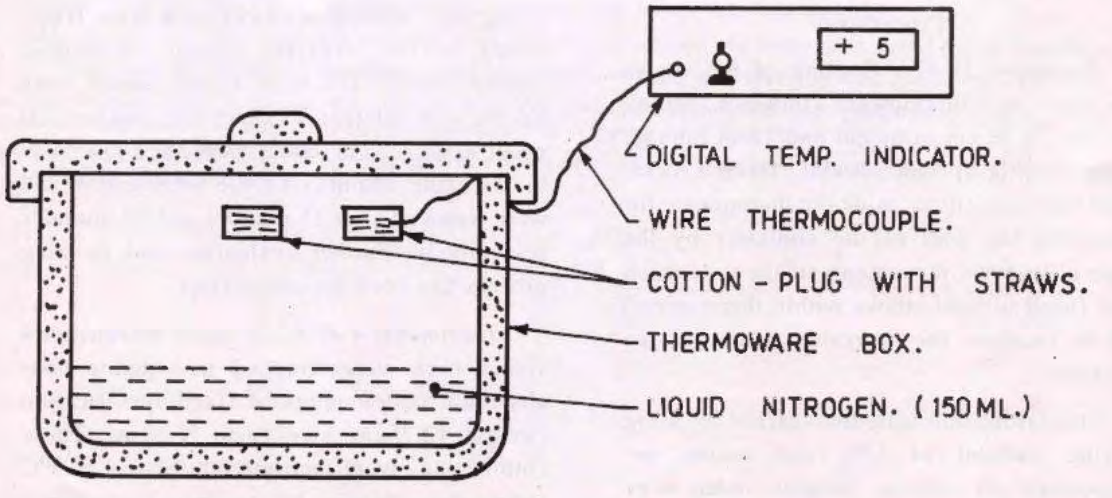

FIG. - DIAGRAMATIC REPRESENTATION OF THE ASSEMBLY USED FOR COOLING SEMEN - STRAWS WRAPPED IN COTTON - PLUG IN LIQUID NITROGEN VAPOUR.

container (Milton cool pack (R), 5 lit. capacity) measuring 25cm (L), 20cm(H), 10cm(W)and having fixed amount (150 ml.)of LN_2 (after priming). Justlike in T-1 the standardization for the height and amount of LN2 was done before start of the experiment. Small rubber bands were used over cotton plug to firmly secure cotton over the straws (Fig.2).

Thermoware was primed with 20-30 ml. of LN2 immediately before use. 150 ml of LN2 was then transferred into the thermoware, lid was closed for 2-3 minutes to enable the vapour to stabilise were fitted inside the thermoware at the fixed 5°C mark. The two packets were taken out after 15 mts for the treatments 4 & 5 respectively. Straws were later evaluated and frozen by the common method as described later. The temperature outside the packet after end of 15 mts (T4) and 30 mts (T5) was 2°C (±1°C). Preference was given to time (15 mt. or 30 mts.) irrespective of temperature gradients.

At the end of prescribed period of different treatments, the straws were removed. Out of the 4 straws per treatment, one straw was used for assessment of prefreeze motility while the remaining three straws were frozen by the two-stage cooling method (Jondet *et al*, 1980).

All the straws were thawed at one time at 37°C for 30 seconds. Out of the remaining 3 straws, one was used for assessing post-thaw motility, second straw was kept in incubator at 37°C for one hour for assessing post-incubation motility. The remaining third straw was kept in thawing water in refrigerator at 5°C for 24 hours for assessing post-ageing motility.

Analysis of the data was done on micro-32 computer according to Steel & Torrie (1981).

Results

The mean values for different seminal characteristics were : initial motility 64.00± 2.08%; sperm concentration 712.00±93.91 millions/ml; dead sperm 25.73 ±3.35% and abnormal sperm 10.40±0.41%. In general, there were non-significant differences between treatments for the prefreeze motility. The interaction (bull x treatment) values were also not significent at all the four stages.

Discussion

The non-significant differences between five treatments for the prefreeze motility gave the encouaging indication that cooling to 5°C was possible in LNV without having any adverse effect on sperm motility was even higher for the treatments involving cooling by freezing chamber method (T-2 & T-3). But, at the same time the values were lowest for the treatments involving cooling of cotton plug protected straws in LNV(T4 & T5).

For the post-thaw, post-incubation and post-ageing motility the values were again highest (like prefreeze motility) for T1 which involved cooling to 5°C in LNV in 15 mts. Freezability was better for the treatments 1,5 and 4 which involved cooling to/at 5°C in LNV as compared to treatments which involved cooling to 5°C in freezing chamber or the refrigerator(T3 & T2).

These observations indicate that LNV can be used in cooling the semen from initial temp. of 30°C to 5°C. The desired cooling time can perhaps further be modified as per the need by use of programmable freezer. It can replace the need of refrigerator and the entire freezing process can be completed with the help of LN_2 only. Such a complete protocol need to be further worked out commercially which can also be used for

custom/freezing and for the centres where supply of electricity is erratic. Such conditions are common especially at the freezing stations located in rural or semi-urban areas.

The study further confirmed the observation made in our earlier experiments (Mathur, 1989)that straws immediately after filling and sealing could be placed directly to 10°C, then could be cooled to 5°C, instead of cooling from around 30°C.

Table 1 : **Mean motility values after different cooling treatments at different stages of processing/evaluation.**

State of motility assessment	Mean motility values (%) for different treatments				
	T-1	T-2	T-3	T-4	T-5
1. Prefreeze stage	57.00 ± 3.33	56.66 ± 2.56	57.33 ± 2.33	51.66 ± 3.89	44.33 ± 4.19
2. Post-thaw stage	32.33 ± 3.80	21.33 ± 3.82	21.66 ± 2.32	26.80 ± 3.90	29.00 ± 4.63
3. Post-incubation stage	15.66 ± 3.19	6.33 ± 2.15	7.66 ± 1.59	10.13 ± 3.18	10.33 ± 2.81
4. Post-ageing stage.	15.66 ± 3.51	6.80 ± 1.92	7.93 ± 2.43	8.46 ± 2.36	13.33 ± 3.22

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Testicular Biometry And Seminal Characteristics Of Kangayam Bulls

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Testes size and seminal characteristics vary in different breeds and species. Zebu bulls have smaller testicular size than exotic dairy breeds. Evaluation of breeding soundness in any breed requires accurate knowldge about normal testicular size and semen characteristics in that particular breed. Reports are scanty on biometry of testes and seminal

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characteristics in Kangayam breed (Pattabiraman, 1958; Appadurai, 1971).

Materials and Methods

Sixteen Kangayam bulls aged 46 to 119 months, stationed at 3 bull stations and one Livestock farm in Tamil Nadu constituted the material for this study . Semen was collected from these bulls twice a week regularly for use in AI Programme. The bulls were divided age wise in three groups.

Group	Age (Months)	No.of animals
I	< 60	5
II	60 – 84	5
III	> 84	6

These bulls were apparently normal and in good health. Scrotal circumference was taken at the maximum point as per Ball *et al* (1983). Testicular length and width were recorded as per Hahn *et al.* (1969). Two ejaculates on three occasions were evaluated as per Ball *et al,* (1983).

Taable 1 : **Biometry of testes and seminal characteristics in different age groups of Kangayam Bulls (Mean ± SE)**

Sr. No.	Parameters	Gr. – I	Gr. – II	Gr. – III
1	Scrotal Circumference (cm)	27.60 ± 1.47 [a]	30.60 ± 1.29 [b]	32.08 ± 0.67 [b]
2	Left testes – Length (cm) – Width (cm)	10.60 ± 0.40 [a] 5.40 ± 0.29 [a]	10.30 ± 0.47 [a] 5.90 ± 0.33 [a]	10.75 ± 0.31 [a] 6.08 ± 0.08 [a]
3	Right testes – Length (cm) – Width (cm)	10.60 ± 0.40 a 5.40 ± 0.29 a	10.40 ± 0.43 [a] 5.90 ± 0.19 [a]	10.50 ± 0.45 [a] 5.92 ± 0.16 [a]
4	Seminology			
	(i) Volume (ml)	3.55 ± 1.22 [a]	5.28 ± 0.61 [a]	4.66 ± 0.60 a
	(ii) Initial Motility (%)	62.00 ± 3.74 [a]	60.00 ± 4.08 [a]	59.00 ± 5.10 [a]
	(iii) Concentration (Millions/ml)	1092.00 ± 109.05 [a]	1350.75 ± 279.03 [a]	1363.60 ± 122.00 [a]
	(iv) Live sperm count %	85.32 ± 4.71 [a]	81.87 ± 5.32 [a]	80.13 ± 7.02 [a]
5	Sperm morphology			
	(i) Primary Abnormality	3.80 ± 1.59 [a]	3.73 ± 0.74 [a]	1.92 ± 0.46 [a]
	(ii) Secondary Abnormality	6.20 ± 1.02 [a]	8.43 ± 2.46 [a]	9.00 ± 1.98 [a]
	(iii) Total abnormality	10.00 ± 1.79 [a]	12.15 ± 3.18 [a]	10.92 ± 1.96 [a]

Differences between groups in all parameters except scrotal circumference were not statistically significant

Results and Discussion

The mean scrotal circumference in Group-I was significantly lower than other age groups. (Table 1). Coulter and Foote (1977) reported that the scrotal circumference increased with age groups. The mean scrotal cirumference in all age groups was less than the values reported in exotic dairy and beef breeds (Coulter *et al,* 1975; Ball *et al,* 1983).

There was no significant differnence in testicular length and width and seminal characteristics between different age groups, Testicular length and width are in agreement with the observations of Appadurai (1971) in Kangayam breed but lower than the values reported in other Indian breeds (Sane *et al,* 1982). The mean mass activity was found to be lower and volume, concentration live sperms percent and total sperm abnormality recorded in this study were more or less similar to the observations in other important (Haryana, Tharparkar and Ongole) Indian breeds (Tomar *et al,* 1964; Rao & Rao, 1975, 1980). It is suggested that while evaluating Kangayam bulls for breeding soundness, the smaller testicular size, characteristic of this breed should be kept in mind, in comparison to exotic bulls.

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Studies On Cross-bred Boar Semen Characteristics And Preservation.

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Piggery development is taken up on large scale by introducing exotic breeds for genetic improvement in the native population. Literature on the semen characteristics and preservation of semen of cross-breed boars is scanty. Hence, a study on various semen characteristics of crossbred boars was taken up. Simultaneously a comparison was made on the efficacy of certain diluents in preserving the semen at two different temperatures viz. 5°C and 15°C.

A total of 72 collections were taken from 6 F_1 boars [Desi sow x Large White Yorkshire (LWY) boar], belonging to the All India Coordinated Research Project (AICRP) on Pigs, College of Veterinary Science, Tirupati, by using artificial vagina. Service behaviour and semen characteristics were noted. For preservation studies 72 ejaculates, 4 from each boar collected at weekly intervals and preserved at 5°C and 15°C in four different diluents by split sample techbnique were utilised. The diluents were: Glucose potassium sodium tartarate sodium citrate dihydrate edate (GPSE) (Tamuli *et al.*, 1984); Kiev I diluent; Kiev II diluent and Glycose-glycine- EDTA-sodium bicarbonate-citrate diluent (GGEBC) (Vijayakumaran and Iyer, 1980).

The diluents were prepared aseptically and fortified with 1000 units of penicillin and 1000 ug dihydro-streptomycin per ml of the diluent. The semen was diluted at 1:2 ratio. The progressive motility of sperms was assessed in all the samples immediately after dilution and after 12, 24, 48, 72, 84 and 96 hours of storage.

Reaction time (minutes) 1.58±0.11; duration of ejaculation 3.39±0.10 min, total volume 163.43±8.0 ml; strained volume 133.54±6.11 ml; gel volume 29.89±1.81 ml; initial sperm motility 85.14±0.35%; pH 7.24±0.01; sperm concentration 274.0±9.83 millions/ml and live sperms 83.47±1.64%.

A significant variation was found in the reaction time and strained volume of crossbred boar semen. No significant variation was found in the duration of ejaculation, average gel volume, percentage of progressively motile sperms and pH of semen, between boars.

The reaction time in crossbred boars was shorter as compared to that in Large White Yorkshire (Murthy, 1977), exotic crossbreds (Ugwv *et al.*, 1984) and native boars (Venumanohara Rao, 1989). The average duration of ejaculation in this study was allied in exotic crossbreds (Ugwv *et al.*, 1984) and native boars (Venumanohara Rao, *et al.*, 1989). The mean total volume of ejaculate in crossbreds was allied in the same as in LWY boars (Sreekumaran and Raja, 1976). Similarly, the average strained and gel volumes of crossbreds in the present study were allied to the findings of earlier workers. The mean gel volume constituted nearly 20% of the total volume of ejaculate.

The mean per cent of progressively motile sperms was found to be 85.14±0.35. This is in

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agreement with the report of Murthy (1977) in LWY boars. Ugwv *et al.* (1984) recorded much lower values in exotic crossbreds.

The mean pH of semen recorded in the present investigation was very close to the values reported by Ilinskaya and Bezlyudnikov (1978) in LWY boars and Venumanohara Rao (1989) in native boars. Similarly, the mean sperm concentration (274.0±9.83 millions per ml) in this study is near to the value reported in LWY by Murthy (1977) and native boars (Venumanohara Rao, 1989). But Ugwv *et al.* (1984) reported much lower sperm concentration in exotic crossbred boars.

The live sperms percentage noticed in this study was in close agreement with the findings of Murthy (1977) in LWY boars and Ugwv *et al.* (1984) in exotic crossbred boars.

The average percentage of progressively motile sperms was significantly higher ($P<0.01$) in the samples kept at 15°C than at 5°C at all periods of preservation. The mean progressive sperm motility was found to be superior in GPSE diluent as compared to other three diluents, both at 5°C and at all hours of preservation. In GPSE diluent, the overall motility was 58.01% at 15°C and 31.21% at 5°C. This is much lower compared to the reports of Tamuli *et al.* (1984) and Venumanohara Rao (1989) regarding sperm motility in Landrace and native boars, respectively.

In Kiev I, Kiev II and GGEBC diluents, the mean sperm motility at 15°C was 51.95%, 50.68% and 50.71% respectively. These values were almost similar to those reported by Vijayakumaran and Iyer (1980).

The variation in progressively motile sperm percentage during storage and between individual boars, diluents and preservation times was found to be significant ($P<0.01$). The highest percentage of progressively motile sperms was recorded in GPSE (45.44%) and the least in the Kiev II diluent (38.77%). Slight difference was noticed in the keeping quality of semen stored in the Kiev I and GGEBC diluents.

The interaction due to diluents x preservation times, diluents x temperatures, preservation times x temperatures, diluents x preservation times x temperatures was found to be significant ($P<0.01$).

The GPSE diluent gave better result at 15° C at all times of preservation upto 96 hours as compared to other 3 diluents. Similar findings were recorded by Venumanohara Rao (1989) in native boar semen. The superiority of the diluent is attributed to the presence of tartrate and citrate in addition to EDTA as opined by Venumanohara Rao (1989).

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'Dag' Defect In Two Jersey Bulls (Half- Sibs)

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Blom (1966) first reported a hereditary defect of the sperm midpiece and tail 'Dag' defect in two full brothers. The defect is characterised by the presence of over 40-50% of tails strongly coiled, folded together or split up into fibres in the ejaculates. The defect was also observed by others (Koefoed-Johnsen and Pedersen, 1971; Blom and Wolstrup, 1977). To our knowledge this defect has not been reported in India. 'Dag' defect found in two Jersey bulls (Half Sibs) is reported in this paper.

Materials and Methods

Two Jersey bulls (Nos. 220 and 282) aged 2 years & 8 months located in two farms in Tamil Nadu and born to same sire but two different dams constituted the material for the study. They were normal for breeding soundness. Three ejaculates from each bull were obtained and examined for seminal quality as described by Ball *et al* (1983).

Results

Findings of semen studies of the two Jersey bulls are presented in Table.1

Jersey bull No. 220 was having strongly coiled, folded or broken midpiece and tails (77.1 ± 4.15%) to the extent reported by Blom (1966), Koefoed-Johnsen and Pedersen (1971) and Blom and Wolstrup (1977). The other Jersey bull (No. 282) had strongly coiled, folded and broken midpiece and tail to a lesser extent (20.8± 5.84%), but it had a high incidence of reversed and kinked tails (61.01± 2.70%). The 'Dag' defect in these bulls was further confirmed by the fact that both are half brothers, similar to the reports of Blom and Wolstrup (1977).

Table 1 : **Semen characteristics of Jersey bulls with 'Dag' defect (Mean ± S.D.)**

Sr. No.	Semen Characteristics	Bull No. 220	Bull No. 282
1	Semenology :		
	(i) Volume (ml)	5.67±1.89	5.13±1.60
	(ii) Initial motility (%)	0	0
	(iii) Concentration (millions/ml)	796.7±335.6	1233.3±308.5
	(iv) pH	6.5	—
	(v) Live sperm count (%)	77.5±4.08	72.6±3.06
2	Sperm morphology (%)		
	(i) Proximal droplets	5.5±1.37	1.0±0.95
	(ii) Head abnormalities	1.7±2.89	1.6±1.47
	(iii) Strongly coiled, folded or broken midpiece and tails	77.1±4.15	20.8±5.84
	(iv) Reversed and kinked tails	12.4±0.78	66.1±2.70
	(v) Distal droplets	—	2.3±1.10
	(vi) Separated heads	1	2.2±1.99
	(vii) Total abnormalities	97.7±2.13	94.0±5.86

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Comparative Study Of Certain Enzymatic And Biochemical Constituents Of Epididymis And Vas deferens Between Buck And Boar

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ABSTRACT

The study was conducted on 10 Black Bengal bucks and 10 boars. The enzymatic and biochemical constituents of epididymis and vas deferens studied were glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), acid phosphatase (ACP), alkaline phosphatase (ALP), inorganic phosphorus and protein. These, except inorganic phosphorus were significantly higher in boar than in buck. The levels in both boar and buck were higher in cauda than caput epididymis, except the levels of GPT and protein which were higher in caput region only. The intensity of increase of enzymatic activity particularly in caput epididymis of boar varied 7 to 18 times with protein content 2.5 times higher than that in goat.

* * *

Detailed studies on enzymatic constituents of epididymis and vas deferens of bucks and boars are lacking particularly since buck ejaculates less volume of semen with high concentration of spermatozoa while boar donates large volume of semen with less sperm concentration. Therefore, the present study was undertaken to meet this lacuna.

Materials and Methods

Testes with epididymis and vas deferens of 10 Black Bengal bucks were collected by open method of castration (O'Conner, 1980) and of 10 boars by slaughter. These ware brought to the laboratory in a container maintained at 0°C. The epididymis together with vas deferens of each testis was separated out and kept on a watch glass placed over ice cubes. The caput with corpus and cauda epididymis were placed on two separate watch glasses over the ice cubes. 10% homogenates of head and tail region were made separately in a glass homogeniser with chilled water and centrifuged at 3,000 r.p.m. for 10 minutes. The

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supernatant of each homogenate was divided into two parts. One part was deproteinised with 10% tri-chloroacetic acid (TCA) in ratio of 1:5 and centrifuged at 3000 r.p.m. for 10 minutes. The supernatant was then utilised for the estimation of inorganic phosphorus (Fiske and Subbarow, 1925).

The remaining part of the homogenate was utilised for estimation of glutamic oxaloacetic acid (GOT) and glutamic pyruvic transaminase GPT (Yatazidis, 1960) ; acid and alkaline phosphatase (Hawk *et al.*, 1965) and protein (Wooton, 1974). The data thus obtained were statistically analysed by 't' test as per Snedecor and Cochran (1980).

Results and Discussion

All the enzymatic activities and biochemical constituents except the level of inorganic phosphorus studied, were exceedingly high in the epididymis of boar as compared to buck (Table 1). The intensity of increase of enzyme activities particularly in caput region of epididymis of boar varied 7 to 18 times and protein content 2.5 times higher than that of buck. These differences may be due to species variation.

GOT activities were significantly increased in the caput region of epididymis of both species compared to cauda region, while GPT activities remained unaltered in boar or decreased in buck from caput to cauda region. Transaminase activities are intimately related with gluconeogenesis and are subsequently utilised in energy metabolism of sperm. It is possible that the enhanced gluconeogenesis and energy metabolism is absolutely essential to maintain spermatozoa viable and motile.

The alkaline phosphatase activities were significantly higher in the cauda than caput epididymis in both species. On the contrary, there were no significant differences of acid phosphatase activities in both caput and cauda epididymis in both the species (Table 1). Acid phosphatase is a lysosomic enzyme and any increase in its activity suggests hyperactivity of the cell. Scott *et al* (1963) reported that the synthesis of glyceryl phosphoryl choline (GPC) takes place both in caput and cauda epididymis, though the intensity of synthesis is more in the cauda region. Therefore, synthesis of GPC requires a greater quantity of inorganic phosphorus.

That the highest cellular activity was in cauda region of epididymis can be substantiated by the facts that inorganic phosphorus content was more in cauda than caput region in both the species (Table 1). The intensity of the increase of inorganic phosphorus content in cauda epididymis of boar was only significant. Scott *et al* (1963) observed a gradual increase of acid-labile and total phosphorus content of the reproductive tract from testis to vas deferens and they suggested that a correlation might exist between the increase of phosphorus content and synthesis of GPC.

The protein content of the caput region was significantly higher than cauda epididymis in both the species (Table 1). Conjugated protein has a significant role in the maturation of spermatozoa and enormous quantities have been reported in the epididymis (White and Wales, 1961). The proteins are being secreted by the epithelial cells and the quantity of these cells in caput epididymis is always higher. That may be the possible reason for the significant increase of protein content of caput epididymis in both the buck and boar.

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Table 1 : **Levels of certain enzymatic and biochemical constituents of caput and cauda regions of epididymis of buck and boar.**

Constituents	Buck		Boar	
	Caput	Cauda	Caput	Cauda
Glutamic oxalo-acetic transaminase (µg pyruvic acid/mg protein/hr)	$10.52 \pm 0.29^{(a)}$	$31.77 \pm 0.95^{(b)}$	$95.51 \pm 2.40^{(a)}$	$137.94 \pm 4.02^{(b)}$
Glutamic pyruvic transaminase (µg pyruvic acid/mg protein/hr)	$2.07 \pm 0.10^{(a)}$	$1.38 \pm 0.13^{(a)}$	$37.75 \pm 5.85^{(a)}$	$36.38 \pm 14.02^{(a)}$
Acid phosphatase (µg phos/mg/protein/hr)	$1.08 \pm 0.06^{(a)}$	$0.895 \pm 0.35^{(b)}$	$18.99 \pm 1.98^{(a)}$	$22.01 \pm 1.46^{(a)}$
Alkaaline phosphatase (µg phos/mg/protein/hr)	$3.87 \pm 0.16^{(a)}$	$8.19 \pm 0.52^{(b)}$	$42.86 \pm 2.03^{(a)}$	$84.84 \pm 7.04^{(b)}$
Inorganic phosphorus (µg/gm/protein)	$70.89 \pm 2.7^{(a)}$	$74.76 \pm 1.99^{(b)}$	$11.80 \pm 0.64^{(a)}$	$23.78 \pm 1.78^{(b)}$
Protein (µg/gm tissue)	$19.95 \pm 0.85^{(a)}$	$9.39 \pm 0.39^{(b)}$	$51.10 \pm 1.36^{(a)}$	$29.20 \pm 1.27^{(b)}$

Note : 1. Values having disimilar superscripts vary significantly ($P \angle 0.05$) from each other.

2. Number of observations considered for each parameter in buck and boar-10.

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Age At Puberty, Incidence Of Delayed Puberty And Effect Of Birth Weight, Exotic Inheritance And Season Of Birth On Puberty In Cross Bred Heifers *

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ABSTRACT

Reproductive records of cross-bredheifers maintained at five Livestock Farms in Vidarbha were studied over a ten year (1978 to 1987) period. The avarage age at puberty was 867.45±41.13 days with 64.13% incidence of delayed puberty in crossbred heifers. Heifers with 26 to 30 Kg birth weight reached puberty earlier than those with lower birth weight. The average age at puberty was lowest (852.32± 34.08) in heifers with 50% exotic inheritance, followed by 75% (861.20±35.36 days) and 62.50% (889.71±38.95 days). Heifers born in summer reached puberty at earlier age (841.52±33.01 days) than those in winter (874.77± 38.09 days) and monsoon (884.82± 36.69days).

* * *

Besides augmenting milk production, one of the important objectives of crossbreeding indigenous cattle is to reduce the age at puberty/maturity for obvious economic benefit. However, the success achieved in this respect seems to be questionable. Although the earlier results indicate the desired achievments (Goswami and Datta, 1959; Bhatnagar *et al.*, 1975 and Bhat, 1977), the recent reports, however, suggest that the age of puberty in crossbreds is showing increasing trend (Manickam *et al.*, 1978; Baghel, 1988; Deshmukh and Kaikini,1989). The present work was undertaken to study the age at puberty, incidence of delayed puberty under agro-climatic conditions of Vidarbha besides the effect of birth weight, exotic inheritance and season of birth on puberty of crossbred heifers.

Materials and Methods

Crossbred heifers which had attained adequate body weight (over 200 Kg) and age (above 2 years) but failed to exhibit estrus and on gynaeco-clinical examination had both ovaries smooth and inactive with flaccid and atonic uterus were regarded as delayed pubertal. The average age of puberty and the incidence of delayed puberty were studied from records (1978 to 1987) maintained at University (PKV) Livestock Farms Akola, Borgaon and Nagpur and State Government Dairy farms located at Pohra and Yavatmal.

Results and Discussion

The average age at puberty in crossbred heifers observed in the present study was 867.45± 41.13 days (Table 1). These findings are in agreement with Manickam *et al.* (1978)

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and Deshmukh and Kaikini (1989). However, these are much higher than the reports of Kottaya and Rao (1973), Kaikini *et al.* (1981), Fulsounder *et al.* (1984), Sharma *et al.* (1986) and Shah *et al.* (1989). The higher age at puberty recorded in the present investigation may probably be due to low plane of nutrition which adversely affects the ovarian activity (Grim, 1954). Adequate level of nutrition is necessary for the proper functioning of endocrine system, since it influences synthesis as well as release of the hormones from the endocrine glands. The proper growth and development of reproductive organs of the immature female is retarded by under nutrition, regardless of whether there is low level of energy, protein, minerals or vitamins. Similarly, mineral and trace element deficiencies which also greatly influence the onset of puberty might have acted as contributory factors in the present study. Animals in India receive only 27% requirements of digestible protein and 44% of their energy requirements from available feeds. It is, therefore, possible that they suffer from single or multiple deficiencies which lead to slow growth and inefficient utilization of food (Dutt, 1972). The higher age at puberty may also be due to higher atmospheric temperature specially during summer.

The overall incidence of delayed puberty in these farms of Vidarbha region averaged 64.13%. However, highest overall incidence (98.30%) was recorded in C.B.F. Borgaon and lowest (38.66%) in Dairy Farm, Yavatmal. The present findings are in agreement with Lagerlof (1954) who reported 61.8% incidence of subfunctional ovaries in Indian cows. However, the present incidence is much higher than report (15 to 20%) of Gibbon (1954), 18.30% by Kodagali (1968) and 15 to 20% anestrus/delayed puberty by Kaikini *et al.* (1977). Baghel (1988) also reported lower incidence of delayed puberty (20.51%) in crossbred heifers. The variation in incidence at various farms may be due to difference in managerial practices, especially of the growing heifers. Besides the temperature fluctuations with hot summer and longer dry spell leading to nutritional and climatic stress on heifers might be contributory factors for the delay. Anestrus resulting in delayed onset of puberty in heifers has been recognised as the most common form of infertility encountered in cattle (Iyer, 1978). Higher incidence in PKV Farms may be due to nutritional deficiency during growth of heifers, whereas, lower incidence in State Govt. Farms may be due to culling of delayed pubertal heifers.

Table 1 : **Effect of weight at birth, exotic inheritance and season of birth of heifers on age and puberty.**

Factor		No.of Observations	Average (±S.E.) age at puberty (days)
Weight at birth (Kg)	11-15	68	876.73 ± 37.84
	16-20	232	875.06 ± 34.32
	21-25	134	854.88 ± 36.89
	26-30	34	844.44 ± 35.31
Exotic inheritance (%)	50-00	197	852.32 ± 34.08
	62.50	157	889.71 ± 38.95
	75.00	114	861.20 ± 35.36
Season of birth	Winter	193	874.77 ± 38.09
	Summer	143	841.52 ± 33.01
	Monsoon	132	884.82 ± 36.69

Females with 26 to 30 Kg birth weight reached puberty at an earlier age than other groups of females with lower birth weight (Table 1). The overall average age at puberty for crossbred heifers with 50%, 62.50% and

75% levels of exotic inheritance was found to be 852.32± 34.08, 889.71±38.95 and 861.20± 35.36 days respectively, which is in close agreement with the findings of Deshmukh and Kaikini (1989). Females born in summer reached puberty at earlier age (841.52±33.01 days) than those in winter (874.77±38.09 days) and monsoon (884.82±36.69 days). Females born in summer reached puberty at earlier age, possibly due to favourable climatic conditions of monsoon and winter resulting in faster growth. However, there was no significant effect of birth weight, exotic inheritance and season of birth on age at puberty.

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Studies On Gravid Uteri Of Buffaloes

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ABSTRACT

Observations on intact gravid uteri and gonads were recorded. Out of 36 uteri, 21 (58. 33%) showed right horn and 15 (41.67%) left horn pregnancy. Increase in weight of intact gravid uteri was highly significant (P< 0.01) at various stages of pregnancy. All linear measurements of gravid uterine cornua were found highly significant (P<0.01) while in non gravid uterine cornua, circumference was highly significant (P<0.01) and greater curvature was significant at different stages. The weights of ovaries and corpora lutea vera varied non-significantly at various stages of pregnancy.

* * *

The knowledge of the morphometric changes likely to occur in the gravid uteri at various stages of pregnancy is essential for breeding operations and also to know the stage of abortion. Proper understanding of size variations during normal pregnancy is of importance to arrive at accurate differential diagnosis. Hence an attempt is made to establish the norms of gravid uteri of buffaloes at different stages of pregnancy.

Material and Methods

The present study was carried out on 36 gravid uteri of slaughtered buffaloes at various stages of pregnancy irrespective of breed, age and nutritional status. The extraneous tissues were dissected before recording biometrical measurements. Gross examination of the intact gravid uterus was undertaken and weights and masurements of various components including ovaries were recorded adopting the techniques of De-Lange (1950) and Sisson and Grossman (1975).

Stage of pregnancy was determined according to Soliman's formulae (1975). According to the stage of pregnancy,the gravid uteri were grouped in 9 different stages beginning with first stage of 30 to 60 days followed by fortnightly intervals upto 165 days and last stage between 166 and 210 days using completely randomised design (CRD) as per Snedecor and Cochran (1967).

Results and Discussion

1. *Weight of Gravid Uterus :* The weight of the uterus including both gravid and non gravid uterine cornua upto 60 days of pregnancy was 815±60.48 g and it increased as the pregnancy advanced . The average weight recorded from 166 to 210 days of pregnancy was 16433.33±151.19 g. There was highly significant increase (p <0.01) in weight at different stages of pregnancy. Similar observations on weight of gravid genitalia were made by Luktuke (1983) and Khan (1989) in buffaloes.

2. Side of Gravidity : Of the 36 gravid uteri, 21 (58. 33%) showed right horn and 15 (41.67%) left horn pregnancy. This indicates that pregnancy occurs more in right horn than in the left in buffaloes. These findings are in agreement with the reports of Kaikini (1974, 1975) in Berari buffaloes and Kaikini and Pankey (1974) who recorded a higher

incidence of right cornual (56.86%) than left cornual pregnancy in buffaloes. Similar findings have been reported by other workers in buffaloes (Anon, 1969; Luktuke, 1983; Ohashi *et al*, 1986; Rind *et al* 1987 and El-Wishy *et al*, 1988). However, Deshpande *et al*, (1985) reported higher incidence of left cornual (55.5%) pregnancy in Surti buffaloes.

3. *Uterine Cornua:* The average length at greater and lesser curvature upto 60 days of pregnancy was 48.97 ± 3.83 and 24.60± 2.19 cm and from 166 to 210 days of pregnancy163.67 ± 1.20 and 45.0 ± 3.60 cm respectively. A significant increase (P< 0.01) in the length at greater and lesser curvatures of gravid uterine cornua during different stages of pregnancy was noticed.

Similarly, circumference of gravid uterine cornua was 18.75 ± 1.13 cm upto 60 days of pregnancy and 88.7 ± 1.88 cm from 166 to 210 days of pregnancy. Highly significant difference (P< 0.01) was noticed in cornual circumference at different stages of pregnancy.

The increase in the length and circumference was due to distension of gravid uterine cornua with its contents of fetus, fetal sacs and fetal fluids.

b) *Non Gravid Uterine Cornua :* The average length at greater and lesser curvatures upto 60 days of pregnancy was 39.97±3.02 and 21.52±1.17 cm and from 166 to 210 days of pregnancy 54.73±0.90 and 23.6±6.30 cm, respectively. Significant difference (P < 0.05) in the length at greater curvature and non significant difference in the length at lesser curvature of non-gravid uterine cornua was noticed at different stages of pregnancy.

Similarly, circumference of non-gravid uterine cornua was 12.85±1.02 cm, upto 60 days of pregnancy and 24.7±3.21 cm, from 166 to 210 days of pregnancy. The measurements were highly significant (p < 0.01) at various stages.

The concurrent increase in the length at greater and lesser curvatures of gravid and non gravid uterine cornua was recorded by Luktuke (1983). Khan (1989) also recorded similar observations.

4. *Ovaries :*

(a) *Cross Pregnancy :* In the present study, only one gravid uterus was having a cross pregnancy out of 36 pregnant uteri. The corpus luteum of pregnancy was present on the ovary corresponding to the non-gravid cornua.The incidence of the transmigration of ovum, was therefore, only 2.78%.

These findings are in full agreement with the reports of Kaikini and Pankey (1974) who recorded an incidence of 2.86% contra-lateral (cross) pregnancies (left to right 1.91% and right to left 0.95%) in 105 buffaloes studied by them. Luktuke (1983) reported a lower incidence (1.67%) of cross-pregnancy in 120 buffaloes studied by him.

(b) *Dimensions and Weights of Ovaries :* Biometry on the 36 pairs of ipsilateral and contralateral ovaries included length, width, thickness and weight at different stages of pregnancy. The average length, width and thickness of the ipsilateral and contralateral ovaries in relation to gravid uterine cornua upto 60 days of pregnancy was 2.5±0.93, 2.28±0.19, 1.42±0.11 and 2.33±0.17, 1.85± 0.14, 1.2±0.06 cm, respectively. The corresponding dimensions between 166 and 210 days of pregnancy were 3.4±0.25, 2.27±0.13, 1.43±0.12 and 3.03±0.18, 1.77±0.30, 1.1±0.06 cm, respectively.

Average weights of the ovaries upto 60 days of pregnancy were 5.54±0.46 and 3.34

± 0.55 g,, and that from 166 and 210 days of pregnancy were 4.47±0.33 and 2.50±0.36 g, respectively.

The increase in mean weight of ipsilateral ovaries was more when compared with those on contralateral non-gravid cornua at different stages. This could be attributed to pregnancy corpus luteum which was present on ipsilateral ovaries. No significant difference was observed in the average weights of ipsilateral and contralateral ovaries at various stages of pregnancy.

Similar observations on dimensions of ovaries were reported by Hafez (1955). Kaikini (1974), Rind *et al,* (1987) and Khan (1989) in buffaloes. Luktuke (1983) recorded lower weights of both the ovaries between 4 and 14 weeks of pregnancy in buffaloes.

5. *Weight of Corpus Luteum (CL) :* The average weight of C.L. verum upto 60 days of pregnancy was 2.33±0.35 g and that from 166 to 210 days of pregnancy was 2.15±0.25 g. No correlation was observed between the stage of pregnancy and the weight of the C.L. verum. Average weight of CL verum was maximum between 91 and 105 days of pregnancy (2.84g). No significant difference was noticed in average weights of CL. vera at various stages of pregnancy.

Similar findings were reported by Luktake (1983) and El-wishy et al, (1988) in buffaloes. However, the present values are much higher than those reported (1.94 gm.) in Berari buffaloes by Kaikini (1974) and Khan (1989) in Nili-Ravi buffaloes, but decidedly low than the findings of Singh *et al,* (1990) buffaloes.

Corpus luteum is essntial for maintenance of premancy to full term in buffaloes, irrespective of non-signifiance recorded in its weight.

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Serum Biochemical Changes Associated With Uterine Torsion In Buffaloes

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The present study was undertaken to estimate total protein,cholesterol,glutamic oxaloacetic transaminase(GOT) and glutamic pyruvic transaminase(GPT) levels in serum of uterine torsion affected buffaloes following surgical correction. Thirteen bufaloes with uterine torsion reported at the University Veterinary Clinics were divided into two groups :

Group I : Six buffaloes in which uterine detorsion was achieved but cervix failed to dilate and caesarean section was performed to deliver the foetus.

Group II : Seven buffaloes in which uterine detorsion was not feasible and caesarean section was performed to deliver the foetus.

Jugular venous blood samples were collected, before detorsion(Gr.I), before and after caesarean section and 24, 48, 72, and 96 hrs. after operation.

The serum protein levels did not show any significant change before and after detorsion, but it declined after caesarean section and 24 hr post-surgery as compared to pre-operation values (Table 1). These values further got elevated at 48, 72 and 96 hr post-surgery but were significantly lower ($p<0.01$) than the values recorded earlier (Singla, 1988) in normally calving animals around parturition. Similar results have also been reported by Khatri et al.(1986) in cases of uterine torsion. The decline in serum protein concentration found in these studies ,may be attributed to the stress of uterine torsion. Marginal decrease in plasma cholesterol concentration was noticed after detorsion. These values further decreased upto 96 hr post-surgery. Similar observations have been recorded by Phogat (1987). There was no significant difference in serum GOT and GPT activities before and after detorsion. However, these values were significantly higher as compared to normally calving buffaloes. Lower levels of serum GOT and GPT after 72 and 96 hr post-surgery indicate recovery of the animal from stress.

Table 1 : **Serum biochemical constituents (Mean ± SE) of uterine torsion cases in buffaloes (n = 13)**

Sr. No.	Time/Period	Protein (gm %)		Cholesterol (mg %)		GOT (IU/Lt)		GPT (IU/Lt)	
		Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
1	Before detorsion	6.38 ± 0.08		165.00 ± 1.84		95.58 ± 2.33		44.38 ± 0.71	
2	After detorsion/ before delivery	6.33 ± 0.08	6.20 ± 0.14	163.00 ± 2.41	164.86 ± 2.42	97.39 ± 0.90	96.56 ± 2.70	43.82 ± 0.80	43.72 ± 0.85
3	After caesarean section	6.10 ± 0.07	5.93 ± 0.14	157.67 ± 2. 38	160.43 ± 2.31	101.26 ± 2.16	98.17 ± 2.43	46.06 ± 0.71	43.78 ± 1.06
4	24 hr. after CS	6.02 ± 0.09	6.01 ± 0.18	153.50 ± 2.62	156.71 ± 2.22	104.79 ± 2.37	105.43 ± 1.44	42.99 ± 0.35	42.02 ± 0.87
5	48 hr. after CS	6.37 ± 0.07	6.31 ± 0.27	150.83 ± 2.40	153.29 ± 1.79	104.16 ± 2.84	103.83 ± 2.98	42.99 ± 0.35	42.11 ± 0.85
6	72 hr. after CS	6.52 ± 0.05	6.73 ± 0.15	150.67 ± 2.85	158.71 ± 1.67	97.94 ± 3.92	100.64 ± 3.53	41.31 ± 0.56	42.11 ± 0.68
7	96 hr. after CS	6.83 ± 0.06	6.90 ± 0.16	149.67 ± 3.30	160.43 ± 1.90	94.42 ± 3.88	98.69 ± 3.74	41.59 ± 0.80	41.59 ± 0.90

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Biometrics Of Fetal Membranes In Buffaloes

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ABSTRACT

Biometrics of fetal membranes in 36 gravid buffaloes were recorded. The placentomes were in four rows with maximum 102 in gravid and 96 in non-gravid uterine cornua. Placentomes in both the uterine cornua were found significant ($P<0.05$) at various stages. Maximum size of placentome in gravid uterine cornua upto 210 days of pregnancy was 10.0x4.6 cm and that in non-gravid uterine cornua 6.6x3.5 cm.

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Fetal membranes play an important role in the regulation of the composition and volume of fetal fluids. Permeability of the membranes to various solutes and hormones such as progesterone, estrogens and prolactin can affect the composition and/or volume of fetal fluid. The knowledge regarding changes in fetal membranes is important for diagnosis, control and treatment of pregnant buffaloes. The present study was therefore undertaken.

Material and Methods

The present study was carried out on 36 gravid uteri of buffaloes slaughtered at Deonar Abattoir, Bombay at various stages of pregnancy.

The biometry on dissected gravid genitalia was recorded following the technique of De-Lange (1950).Uterus was opened with a pair of scissors along the dorsal curvature. Maternal caruncles were separated gently from fetal cotyledons. The intact amnion, chorion and allantois alongwith the embryo/ fetus were separated from the uterus. Stage of pregnancy was determined according to Soliman(1975) formula. The data was analysed by completely randomised design as per Snedecor and Cochran (1967).

Results and Discussion

1. *Shape and pattern of distribution of caruncles* : Placentomes in gravid and nongravid uterine cornua appeared spherical and oval during early pregnancy. During mid and late pregnancy their appearance changed to globular, elliptical or beaded. Placentomes were arranged in more or less prominent rows ranging 4 on an average.

2. *Number of Placentomes* : The average number of placentomes in gravid uterine cornua upto 60 days of pregnancy was 43.83 ±4.96 (27 to 62). Out of these, the average number of caruncles attached and not attached with the cotyledons were 18.5 ± 8.15 (0 to 43) and 25.33 ± 10.44 (2 to 60), respectively. At mid gestation (136 -150 days) the average number of placentomes was 87.67 ± 4.41 (81 to 96) which was maximun with all the caruncles attached to the cotyledons. The average number of placentomes from 166 to 210 days of pregnancy was 66±13.58 (63to90). The maximum number of placentomes in the gravid uterine cornua was 102 from 30 to 210 days of pregnancy.

The average number of placentomes in nongravid uterine cornua upto 60days of pregnancy was 33.5±3.35 (24 to 43). Out of these, the average number of caruncles attached and not attached with the cotyledons were 9.5±6.40 (0 to 37) and 24±7.16 (2 to 43). respectively. At mid gestation (136 - 150 days) the average number of placentomes was 84±12.0 (72to96) which was maximum with all the caruncles found attached with cotyledons. The average number of placentomes from 166 to 210 days of pregnancy was 19.33 ± 7.80 (6 to 33). The maximum number of placentomes in non gravid uterine cornua was 96 from 30 to 210 days of pregnancy.

The average total of placentomes in both gravid and non gravid uterine cornua upto 60 days of pregnancy was 77.83± 7.80 (52 to 102). At mid gestation the same total was 143.67± 30.95 (86 to 192) and from 166 to 210 days of pregnancy it was 85.33± 11.68 (62 to 98). Total number of placentomes in both the uterine cornua was found to be varying significantly ($P<0.05$) at various stages of pregnancy. The number of placentomes in the gravid cornua was in excess of that in the non gravid.

The total number of placentomes increased from early pregnancy to mid pregnancy with

tendency to decrease towards the end of gestation. These findings are in close agreement with those of Hafez (1955), Rajaram and Chandra (1984), Kathiresan *et al* (1988) and Khan (1989) in buffaloes. Roy and Luktuke (1962) and Devaraj *et al* (1979) recorded average number of placentomes in the fetal membrane of gravid and non gravid horns after parturition as 117.8±30.30 (67 to 203) in buffaloes and 98.54 (53 to183) in medium size buffaloes, respectively.

The attachment of the caruncles to cotyledons begins between 28 to 35 days after conception (King *et al*, 1979). In the present study the number of caruncles not attached with the cotyledons upto 60 days of pregnancy ranged from 2 to 60 in the gravid and 2 to 43 in the non-gravid uterine cornua. Between 61 to 90 days of pregnancy, the same number decreased from 18 to nil in the gravid uterine cornua. In the non-gravid uterine cornua the number of caruncles free from the cotyledons from 91 to 105 days of pregnancy decreased from 33 to nil. It clearly indicates that the formation of placentomes in gravid uterine cornua is completed by 90 days and in non gravid uterine cornua by 105 days of pregnancy in buffaloes. Similar observations in buffaloes are not available in literature.

3. *Size of Placentomes* : Maximum size of placentomes in gravid uterine cornua upto 60 days of pregnancy was 1.35 ± 0.26 cm in length (L) and 0.65 ± 0.10 cm in breadth (B). In non gravid uterine cornua, the maximum size was 1.05 ± 0.10 cm L and 0.6 ± 0.09 cm B. There was a gradual increase in their size in both the cornua from 40 to 210 days of pregnancy. From 166 to 210 days of pregnancy, the maximum size of placentomes in gravid and non-gravid uterine cornua measured 8.8 ± 0.92 x 4.83 ± 0.12 cm and 4.07 ± 0.68 x 2.83 ± 0.73 cm, respectively. The dimensions of the placentome varied with the stage of pregnancy as well as their position in the uterus. The placentomes of maximum size in gravid and non-gravid uterine cornua measured 10.0 x 4.6 and 6.6 x 3.5 cm respectively. The large sized placentomes were more in gravid uterine cornua than in non gravid cornua. These were encountered in the mid region of gravid cornua in the vicinity of nmbilical cord, gradually becoming smaller towards the extremities. A consistent increase in the number and size of the placentomes could be attributed to the requirement of more surface area needed for transplacental exchange during pregnancy (Rajaram and Chandra, 1984). There was no evidence of accessory cotyledons in either cornua as reported by Sharma *et al* (1982). Similar observations were made by Hafez (1955), Roy and Luktuke (1962), Rajaram and Chandra (1984). Kathiresan *et al* (1988) and Hradecky *et al* (1988) in buffaloes. Rajaram and Chandra (1979) recorded lower dimensions of placentomes than those reported in the present study.

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Study Of Biometry Of Surti Buffalo Ovaries And Development Of Ovarian Follicles In Relation To Different Seasons

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ABSTRACT

105 pairs of Surti buffalo ovaries were studied for biometry and development of ovarian follicles during Winter, Summer & Monsoon.

Overall mean weight, length, height and width of right ovary was 2.72 ± 0.10 g., 20.92 ± 0.82 mm, 14.76 ± 0.75 mm and 11.76 ± 0.13 mm respectively. These figures for left ovary were 2.54 ± 0.09 g, 19.63 ± 0.46 mm, 13.83 ± 0.47 mm and 10.74 ± 0.58 mm respectively. Height of ovaries was significantly different during seasons and right ovary was significantly wider than the left.

The follicles were grouped according to their diameter viz. 1 to 4, 5 to 8, 9 to 12 and above 12 mm. During all 3 seasons, the right ovary was having more number of developing follicles than the left ovary. Season had no significant effect on the number of developing follicles.

* * *

Many attempts have been made on studies of biometry of genitalia in different breeds of buffalo by various workers (Polding and Lall, 1945; Damodaran, 1958; Luktuke and Rao; 1962; Bhalla *et al*, 1964; Sane *et al*, 1964; 1965; Kodagali *et al*, 1971; Kaikini, 1974; Parkale and Hukeri, 1989). However the seasonal variation in biometry of buffalo ovaries have not been reported eventhough they express seasonal variation in reproductive efficiency. It is generally accepted that the bovine antral follicles grow and regress continuously throughout the oestrous cycle (Donaldson and Hansel, 1965). In this

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comparison, the buffalo does not exhibit uniform reproductive behaviour throughout the year. Pandey and Raizada (1978) reported practical absence of oestrus in buffalo during the hot months of year. Jankiraman and Mehta (1988) found difference in oestrous cycle and oestrus behaviour throughout the year. The present study was designed to provide further information about the follicular development and biometry of ovaries during all seasons in Surti buffaloes.

Materials and Methods

105 pairs of clean, healthy and normal ovaries of adult Surti buffalo were collected from local slaughter house during winter (November-February), summer (March-June) and monsoon (July-October) seasons.

Biometrical observations of ovaries were recorded as per Sane *et al,* (1964). All the measurements were taken with Vernier Callipers and weight on Monopan balance. The follicles present in right and left ovaries were counted and divided according to their diameter: 1- 4, 5- 8, 9- 12 and > 12 mm diameter. The data were statistically analysed as per Snedecor and Cochran (1980).

Results and Discussions

1. *Ovarian biometry* : The overall mean weight of right and left ovaries was 2.72 ± 0.10 g. and 2.54 ± 0.09 g. respectively (Table-1). These findings are in agreement with Kodagali *et al,* (1971). However the mean weight of ovary reported for Murrah buffaloes (Sane *et al,* 1964) and Jaffri buffaloes (Sane *et al,* 1965) is 3.81 ± 0.06 and 4.01 ± 1.52 g. respectively, which is greater than Surti buffaloes. In present study no significant difference was found in ovarian weights.

The mean length of right and left overies was 20.92 ± 0.82 mm and 19.63 ± 0.46 mm respectively (Table 1). No significant difference was observed in length between right and left ovaries or between seasons. This is in agreement with that reported by Kodagali *et al* (1971). However, in heavy breeds like Murrah and Jaffri buffaloes the length of ovaries is greater (Sane *et al,* 1964, 1965).

The mean height of the right and left ovaries was 14.76 ± 0.75 mm and 13.83± 0.47 mm respectively. Significant difference in the height of ovary was observed during monsoon, followed by winter and summer seasons. with that of Kodagali *et al* (1971).

The average width of right and left ovaries was 11.76 ± 0.13 mm and 10.74 ± 0.85 mm respectively (Table 1) which is in close agreement with earlier reports on Surti buffaloes (Buch *et al,* 1970; Kodagali *et al,* 1971). However, the heavier breeds of buffaloes such as Murrah and Jaffri exhibited greater width of ovaries (Sane *et al,* 1964, 19-65). No significant difference was found in width of ovaries due to seasons. Width of right ovary was found to differ significantly from left ovary (Buch *et al,* 1970; Jankiraman and Mehta, 1988), this significant difference in width is evident.

It is concluded that seasons do not affect the biometry of Surti buffalo ovaries except its height. The right ovary was found to be more wider than the left in Surti buffaloes.

2. *Development of ovarian follicles* : The distribution of follicles of different diameters was not significantly related to the seasons. However, stagewise distribution of developing follicles exhibited significant difference between right and left ovaries, especially in case of follicles above 12 mm diameter (Table 2).

The occurrance of large number of follicles (1 to 4 mm) seems to be due to their

faster turnover between development and atresia. Earlier studies in crossbred Herford x Holstein heifers (Matton *et al,* 1981) and cows (Mc-Natty *et al,* 1984) have exhibited similar distribution of ovarian follicles. Matton *et al,* (1981) have shown that most of the follicles with diameter between 2 to 5 mm undergo faster atretic changes as compared to larger follicles. The mean unmber of 1 to 4, 5 to 8, 9 to 12 and >12mm diameter follicles in buffaloe ovaries were 1.34, 0.25, 0.18 and 0.05 per ovary in present study. The lower distribution of ovarian follicles in Surti buffalo as compared to exotic cattle (7.06 ± 1.0) may be due to lower turnover rate of follicles in Surti buffalo. Since season did not exhibit any significant effect on the distribution of follicles, the lower trunover of follicles in buffalo seems to be inherent, which may be the reason for lower superovulatory response in buffaloes than cows.

In the present studies, the right ovary had significantly higher number of follicles above 12 mm diameter as compared to left ovary. The earlier studies (Buch *et al,* 1970) made on 430 ovaries of Surti buffalo indicated greater (58.4%) follicular activity in right ovary than the left ovary (41. 6%). Similar findings have been reported by Jankiraman and Mehta (1988).

The present studies indicate that the mechanism leading to ovulation in Surti buffaloes is influenced by seasons, which is in agreement with earlier reports (Jankiraman and Mehta, 1988).

Table 1 : **Biometry of right and left ovaries of Surti buffaloes in relation to different seasons (Mean ± S.E.)**

Season	N	Weight (g)	Length (mm)	Height (mm)	Width (mm)
Right Ovary					
Winter	35	2.56± 0.07	19.69± 0.73	13.74± 0.60	11.49± 0.48
Summer	35	2.67± 0.18	20.60± 1.00	14.31± 0.65	11.86± 0.41
Monsoon	35	2.93± 0.14	22.49± 0.74	16.23± 0.60	11.94± 0.47
Overall Mean ± SE		2.72± 0.10	20.92± 0.75	14.76± 0.82	11.76± 0.13
Left Ovary					
Winter	35	2.37± 0.08	18.71± 0.64	12.89± 0.55	10.00± 0.27
Summer	35	2.69± 0.21	20.23± 0.78	14.43± 0.70	11.89± 0.49
Monsoon	35	2.57± 0.10	19.96± 0.68	14.17± 0.45	10.34± 0.33
Overall Mean ± SE		2.54± 0.09	19.63± 0.46	13.83± 0.47	10.74± 0.58

N= No.of observations

Table 2 : **Number and percent distribution of ovarian follicles according to their diameter (mm) on right and left ovaries of Surti buffalo in relation to different seasons.**

Season	N	Right ovary					Left ovary				
		1-4 mm	5-8 mm	9-12 mm	>12 mm	Total	1-4 mm	5-8 mm	9-12 mm	>12 mm	Total
Winter	70	56 (76.71)	4 (5.47)	11 (15.06)	2 (2.73)	73	35 (62.50)	14 (25.00)	6 (10.71)	1 (1.78)	56
Summer	70	60 (75.94)	8 (10.12)	6 (7.59)	5 (6.32)	79	34 (72.34)	8 (17.02)	5 (10.63)	0 (0.00)	47
Monsoon	70	44 (61.97)	15 (21.12)	8 (11.26)	4 (5.63)	71	54 (87.09)	5 (8.06)	3 (4.83)	0 (0.00)	62
Total	210	160 (71.74)	27 (12.10)	25 (11.21)	11 (4.93)	223	123 (74.54)	27 (16.36)	14 (8.48)	1 (0.60)	165

Figures shown in parenthesis are in percent.

N=No.of observations.

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Occurrence And Nature Of First Post Partum Estrus In Mehsani Buffaloes

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ABSTRACT

Total 36 normally parturated Mehsani buffaloes were included in the present study. The first post-partum (PP) estrus was observed at an average of 56.72±3.58 days (26 to 107 days) with 91.67% silent and 19.44% anovulatory heats. The season, sex of calf, side of pregnancy, parity, gestational length, birth weight of calf, weight of dam at 10th day post-partum, milk yield for 90 days of lactation and the rate of uterine involution did not significantly affect the occurrence of first post-partum estrus.

* * *

Prolonged post-partum service period is one of the most important reproductive limitation in buffaloes. Prolonged inhibition of pituitary by progesterone from corpus luteum (CL) or placenta leading to refractoriness from GnRH is presumed to cause delayed post-partum ovarian activity. This may also be due to the unobserved/silent heats during post-partum period. Rao *et al.*(1973) reported the first post-partum estrus interval in buffaloes to be 125.73 days, whereas Devraj (1982) found it only 28.30 days in Surti buffaloes. Mehsani buffalo is one of the major breed in Gujarat.Practically, no information is available regarding the occurrence and nature of first post-partum estrus in this breed.

Materials and Methods

Total 36 adult, healthy, normally calved buffaloes between 1st to 10th lactation were used for the study. They were maintained at an optimal level of feeding and management at the University Livestock Research Station. Buffaloes were hand milked twice daily in milking byre and calves were allowed to suckle the dam for 5 to 7 minutes prior to milking. Gynaeco-clinical examination was carried out at two days interval beginning from 5th day post-partum, till complete involution of uterus and thereafter at regular intervals till the first post-partum estrus was noticed. These buffaloes were observed daily for external symptoms of estrus and considered to be in estrus from these criteria :

(1) Presence of behavioural signs of estrus like bellowing, mounting,(2) Presence of mucus discharge,(3) Hyperaemia of vaginal mucus membrane, (4) Good uterine tone and (5) Presence of graafian follicle on the ovary.

The buffaloes which did not show any behavioural signs of estrus were classified under 'silent heat'. Those detected in first post-partum estrus were also examined 10th day post estrus, for the presence or absence of CL to confirm ovulation.

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The interval from parturition to first heat was analysed for the effects of season, sex of calf, side of pregnancy, parity, gestational length, birth weight of calf, weight of buffalo at 10th day post-partum and milk yield for 90 days, post-partum.

Results and Discussion

Appearance of first post-partum estrus varied from 26 to 107 days, with overall mean of 56.72 ± 3.58 days. Maximum buffaloes (55.56%) exhibited signs of post-partum estrus between 31 to 60 days, followed by 25% during 61 to 90 days and 11.11% after 91 days, post-partum. Out of the 36 buffaloes examined for occurrence of first post-partum estrus, 3 (8.33%) manifested normal detectable behavioural symptoms of estrus, while the remaining 33 (91.67%) had silent heats (Table 1).

Table 1 : **Number of Animals showing different nature of first post-partum estrus in mehsani buffaloes. (n=36)**

NATURE	Number of Animals	Percentage
(a) SILENT HEAT	33	91.67
i) Ovulatory	26	78.79
ii) Nonovulatory	7	21.21
(b) ESTRUS WITH BEHAVIOURAL SIGNS	3	8.33
i) Ovulatory	3	100.00
ii) Nonovulatory	–	–

These findings are very close to the interval of 56 days, obtained by Usmani *et al,* (1985) in Nill-Ravi buffaloes, but do not agree with Singh *et al,* (1979) who reported an average of 37.78 ± 2.01 days, from parturition to first succeeding heat, with 27.55% silent heat in Murrah buffaloes. Butchaiah *et al,* (1975), Ahmad *et al,* (1983) and Obireddy *et al,* (1986) found longer first post-partum estrus interval than recorded in the present study. McDonald (1980) opined that first post-partum ovulation occurred 25 to 30 days post-partum without any apparent signs of estrus. Luktuke and Roy (1964) observed the first post-partum estrus at 116 days, with only 14.6% silent heats in buffaloes.

The increased incidence of silent estrus may be due to overlooking of short estrus or weak estrual behaviour. This may also be due to paucity of sufficient secretion of estradiol by follicle or due to lack of prime concentration of progesterone to potentiate the action of estrogen in exhibiting the signs of estrus. Hereditary predisposition of this breed to silent heat cannot be ruled out. Estimation of gonadal hormones (progesterone and estradiol) and gonadortophin hormones during post-partum period may also throw more light in this respect.

29(80.56%) cases had ovulatory first post-partum estrus with palpable CL at 10th day post-estrus, while remaining 7(19.44%) had non ovulatory estrus. Further, all animals which failed to ovulate had silent heat.

In the study of Singh (1982), 69.23% buffaloes came in heat within 25 to 30 days post-partum, of which 55.56% were anovulatory and 75% silent heats. Luktuke and Roy (1964) reported only 6.5% first post-partum, anovulatory heats. El. Sheikh and Mohmed (1976) observed 28.43% first post-partum anovulatory estrus in Egyptian buffaloes.

According to Moller (1970), insufficiency of luteinizing hormones is mainly responsible for post-partum anovulation in milch cows. This may be true for the buffaloes, also.

Difference in observations recorded by various authors may be due to methods used for detection of post-partum estrus and managemental practices. Further, the

differences may be due to genetic factors (Kallef, 1938).

Factors Affecting The Occurrence Of First Post-partum Estrus : In the present study, various factors like season, sex of calf, side of pregnancy, parity, gestational length, birth weight of calf, weight of animal at 10th day post-partum and milk yield of 90 days post-partum had no significant effect on the occurrence of first post-partum estrus (Table 2).

However, the period of occurrence of first post-partum estrus was shortest (49.52 ± 3.76 days) during monsoon and longest (69.4 ± 7.52 days) during summer season. Likewise the primiparous and buffaloes in three or more lactations manifested first post-partum estrus around 51.85 ± 4.30 and 51.68 ± 5.48 days, respectively, whereas the animals in second lactation had longer duration (65.53 ± 6.51 days) without any significant difference.

El. Sheikh and Mohamed (1976) observed nonsignificant effect of season on interval from calving to first heat in Egyptian buffaloes. However, they noted that pluriparous buffaloes exhibit first PP heat significantly earlier than the primipara. Singh *et al,* (1979) and Usmani *et al,* (1985) also opined that season of calving did not significantly affect the occurrence of first PP estrus. But Capitan and Takkar (1988) observed significant effect of season on the first PP estrus. Devraj (1982) observed the nonsignificant relationship between parity and appearance of first PP estrus. Obireddy (1986) observed significant effect of parity order on the onset of PP heat in Murrah buffaloes. Singh (1982) revealed the nonsignificant effect of body weight after parturition and milk yield of first trimester of lactation on occurrence of first PP estrus. However, Quayam *et al,* (1987) found that body weight during

Table 2 : **Least square analysis of variance for occurrence of first post-partum estrus in Mehsani buffaloes. (n=36)**

Sources of Variance	d.f.	s.s.	m.s.s.	'F'
Season	2	2814.38	1407.19	3.18
Sex of Calf	1	62.72	62.72	< 1
Side of pregnancy	1	266.78	266.78	< 1
Parity	2	1581.69	790.85	1.79
Gestational Length	1	255.99	255.99	< 1
Birth Weight of Calf	1	146.74	146.74	< 1
Weight of Animal (At 10th Day Post-Partum)	1	3.87	3.87	< 1
Milk yielded for 90 days post-partum	1	0.58	0.58	< 1
Error	25	11048.47	441.94	

post-partum period was significantly related with the onset of first PP estrus in Murrah buffaloes.

Average involution period of uterus in Mehsani buffaloes in the present study was 26.44± 0.70 days, post-partum. The correlation and regression of involution period of uterus and first post-partum estrus was 0.30 ± 0.18 and 1.55 ± 0.93 days, which were statistically nonsignificant. It shows that the post-partum estrus in Mehsani buffaloes is independent of the involuting uterine state. Similar findings were also observed by Bhalla et al, (1966), Butchaiah et al, (1975) and Singh (1982) in Murrah buffaloes and Dhanani et al, (1988) in Kundi buffaloes of Pakistan. However, Peter et al, (1987) revealed the positive correlation ($r=0.502$, $p < 0.01$) between days required for occurrance of first PP estrus in Murrah buffaloes. Mehsani buffaloes exhibiting ovulatory estrus took significantly less time for organs to return in pelvic cavity ($p < 0.05$), involution of nongravid horn ($p < 0.01$) and for complete involution of uterus and cervix ($p<0.01$) as compared to the nonovulatory estrus animals.

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Studies On Fetal Fluids Of Buffaloes

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ABSTRACT

Volume, color, consistency and pH of allantoic and amniotic fluids of buffaloes were recorded during different stages of pregnancy. Up to 105 days of pregnancy, the quantity of allantoic fluid was more than amniotic fluid. Between 121 to 165 days of pregnancy amniotic fluid was more than allantoic and beyond 166 days the allantoic fluid exceeded the amniotic fluid. Both fluids were clear and colorless during early pregnancy. Thereafter allantoic fluid became mucoid and straw colored whereas amniotic fluid became amber yellow, mucoid and syrupy at later stages. pH remained alkaline in both the fluids between 30 and 150 days of pregnancy. Average weights of empty uterus and placenta were also recorded and found to be highly significant ($P<0.01$) at various stages of pregnancy.

* * *

Fetal fluids are important in the efficient handling of fetal waste products and in preventing mechanical shock to the developing fetus during entire gestation. The knowledge regarding changes in fetal fluids is important for diagnosis and treatment of hydrallantois and hydramnios which are common in buffaloes. This study was therefore undertaken.

Material and Methods

The present study was done on 36 gravid uteri of buffaloes slaughtered at Deonar Abattoir, Bombay at various stages of pregnancy.

Uterus was opened with a pair of scissors along the dorsal curvature. Maternal caruncles were separated gently from fetal cotyledons. The intact amnion, chorion and allantois alongwith the embryo or fetus were separated. Fetal sacs were punctured and observations on the allantoic and amniotic fluids were recorded. Stage of pregnancy was determined as per Soliman (1975). The data was analysed as per Snedecor and Cochran (1967).

Results and Discussion.

1) *Volume* : The volume of allantoic and amniotic fluids was recorded separately at various stages of pregnancy. To compare volumes of allantoic and amniotic fluids at various stages, the Student's 't' test was applied.

The average volume of allantoic and amniotic fluids upto 60 days of pregnancy was 72.25 ± 27.58 and 28.7 ± 6.41 ml, respectively (Table 1) with non significant difference. The fetal fluids increased in the early stages of pregnancy at a faster rate. Upto 105 days of pregnancy, the allantoic fluid was clearly in excess of the amniotic fluid. From 106 to 120 days of pregnancy the difference was non significant but from 121 to 165 days of pregnancy the amniotic fluid increased more rapidly than the allantoic with a highly significant difference ($P<0.01$) in volume. From 166 days onward again the allantoic fluid had taken ride over the amniotic fluid with significant difference ($P<0.05$) in volume. From 166 to 210 days of pregnancy the

average volume of allantoic and amnotic fluids recorded was 2253.5 ± 129.96 and 983.5 ± 361.08 ml, respectively. There was significant difference (P<0.01) in the volume of both allantoic and amniotic fluids at various stages of pregnancy. There was also highly significant difference (P<0.01) in total volume of both the fetal fluids at various stages. Similar trend was recorded by Wright (1950), Hafez and Kamal (1955), Arther (1957), Kaikini (1974) in Berari buffaloes, Soliman (1975) and Luktuke (1983).

The variation in the volume of allantoic and amniotic fluids may be as a result of osmosis of the fluid from one sac to the other. During late pregnancy the ingestion and absorption of fluids by gastro-intestinal tract of the fetus may play a part in the regulation of volume of the fetal fluids.

2. *Color and consistency of fetal fluids* : Allantoic and amniotic fluids were clear and colorless during early pregnancy. In mid pregnancy allantoic fluid became straw colored and amniotic fluid amber yellow in color. Towards the end of mid pregnancy both the fluids were like pale yellow urine.

Allantoic and amniotic fluids were watery in consistency during early pregnancy. As the pregnancy advanced, the amniotic fluid became viscid and glairy because the bladder sphincter prevented further release of urine into the amniotic cavity. The probable source

Table 1 : **Biometrics of fetal fluids (N=36)**

Sr. No.	Stage of gestation (days)	Statistics	Allantoic fluid vol. (ml)	Amniotic fluid vol. (ml)	Total Fetal fluid vol (ml)	Weight of uterus (g)	Weight of placenta (g)
1	30 – 60 (n=6)	Range Mean S.E.	15.0 – 165.0 72.25 27.58	3.20 – 38.0 20.7 0.41	18.20 – 198.0 92.95 33.29	500 – 1000 716.67 79.23	8.65 – 100.20 47.47 12.39
2	61 – 75 (n=10)	Range Mean S.E.	41.0 – 310.0 166.6 21.49	36 – 169 82.0 13.18	121 – 426 248.6 28.09	300 – 1000 567 60.33	40 – 200 97.73 16.44
3	76 – 90 (n=3)	Range Mean S.E.	320 – 400 361.67 23.15	270 – 355 308.33 24.89	590 – 755 670 47.70	600 – 900 716.67 92.80	150 – 280 193.33 43.33
4	91 – 105 (n=2)	Range Mean S.E.	470 – 520 495.0 25.0	350 – 365 357.5 7.5	835 – 890 852.5 17.5	900 – 920 910.0 10.0	160 – 210 185.0 25.0
5	106 – 120 (n=2)	Range Mean S.E.	486 – 515 500.5 14.5	400 – 450 425.0 25.0	886 – 965 925.5 39.5	890 – 1000 945.0 55.0	200 – 320 260.0 60.0
6	121 – 135 (n=4)	Range Mean S.E.	427 – 470 446.5 10.65	960 – 1200 1101.0 52.34	1390 – 1659 1547.5 56.72	1050 – 1450 1256.25 85.62	240 – 310 281.25 15.59
7	136 – 150 (n=3)	Range Mean S.E.	750 – 880 800.0 40.41	2800 – 2970 2881.67 49.19	3570 – 3850 3681.67 85.65	1600 – 1990 1746.67 122.52	550 – 750 633.33 60.09
8	151 – 165 (n=3)	Range Mean S.E.	1510 – 1680 1603.33 49.77	3225 – 3870 3608.33 195.88	4845 – 5550 5211.67 204.00	2350 – 2700 2450.0 125.83	800 – 1230 943.33 143.33
9	166 – 210 (n=3)	Range Mean S.E.	2000 – 2430 2253.5 19.96	350 – 1600.5 983.5 361.08	2780 – 3600.5 3237.0 241.42	2500 – 4000 3466.67 484.19	1100 – 1600 1333.33 145.30

of a viscid or mucoid amniotic fluid was saliva and secretions of the nasopharynx of the fetus. After 200 days of pregnancy allantoic fluid was mucoid acting as a natural lubricant for easy passage of the fetus through the birth canal during parturition. The amniotic fluid became slippery mucoid and syrupy in consistency at this stage. Similar observations were made by Hafez (1955), Arther (1965), Roberts (1971), Kaikini (1974) in Berari buffaloes and Soliman (1975).

3. *pH of fetal fluids* : pH of allantoic fluid varied between 7.28 and 7.43 and that of amniotic fluid between 7.37 and 7.46 from 30 to 150 days of pregnancy. According to Soliman (1975), the pH of amniotic fluid remained alkaline throughout gestation, whereas the pH of allantoic fluid changed from alkaline to acid from 240 days to full term. He attributed this change to increased quantity of urine metabolic products from fetus in the fluid.

4. *Weight of empty uterus* : The average weight of empty uterus upto 60 days of pregnancy was 716.67 ± 79.23 g which increased as the pregnancy advanced. The average weight from 166 to 210 days of pregnancy was 3466. 67 ± 486.19 g. During different stages of pregnancy the increase in weight was highly significant ($P<0.01$). Luktuke (1983) reported similar observations on weight of empty uterus in buffaloes upto 100 days of pregnancy.

5. *Weight of placenta* : Average weight of placenta upto 60 days of pregnancy was 47.47 ± 12.39 g. From 166 to 210 days of pregnancy the weight was 1333.33 ± 145.30 g. The difference was highly significant ($P < 0.01$).

Hafez (1954) recorded similar observations on weight of placenta upto mid pregnancy in buffaloes. Luktuke (1983) recorded higher values on weight of placenta.

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Studies On Hormonal And Non-Hormonal Treatments For Summer Anoestrus In Buffaloes.

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ABSTRACT

The efficacy of Fertivet (non-hormonal) treatment and SMB-implant (hormonal) treatment in inducing oestrus in buffaloes during summer was studied in 19 and 36 buffaloes respectively. 5 buffaloes were kept as control.

All (100%) buffaloes treated with SMB implant came in heat. Of these 41.66% conceived to first service, 11.11% to second and 16.66% to third service with overall 69.4% C.R.

In Fertivet treated group, 68.42% buffaloes expressed oestrus, of which 61.53% conceived to first service, 15.3% to second service with over all 84.10% CR.

Oestrus was better induced with Synchromate-B, whereas the conception rate to first service better with Fertivet.

* * *

Environmental factors assume prime importance in fertility patterns of buffaloes, since season shows marked influence on their reproductive performance. Maximum calvings occur during October to December and they go anoestrus during summer. 60 to 65% of rural buffaloes are anoestrus affecting the economy of buffalo breeders (Rao and Rao 1981). It was, therefore, felt necessary to treat this anomaly with regimens which have proved effective for treating buffaloes with long standing anoestrus in summer season.

Materials and Methods

Sixty buffaloes 4 to 10 years of age, with history of long standing (4 to 42 mths) anoestrus gynaeco-clinically examined twice at 10 to 12 days interval and confirmed as true anoestrus were randomly divided in three groups.

Group I comprising of 19 buffaloes were treated with Fertivet (FVT 300) one tablet per day for five days after a drench of 100 ml of 1% Copper sulphate solution. Each tablet of Fertivet 300 (Ar-Ex Labs., Bombay) contains 180 mg. of clomephene citrate and 120 mg. of trans-clomephene. Group II consisting of 36 buffaloes were treated with Synchromate-B ear implant(Intervet-Boxmeer,Holland) containing 3 mg. norgestomate and 5 mg. estradiol valerate followed by 2 ml. I/m injection. The ear implant remained in situ for 10 days. On the 11th day of implant removal the animal was given 600 I.U. of PMSG Chronegest I/m. Group III of 5 buffaloes served as control. The study was carried out over a four months summer period (February to May 1990), at Akola.

Heat Detection was carried out by parading aproned buffalo bull during the

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cooler hours of the day (5 to 6 AM.) and late evening (7P.M.) The intensity of oestrus was classified according to behavioural symptoms and gynaeco-clinical findings.

Buffaloes in oestrus were bred by A.I. with frozen semen or natural service.

Results and Discussion

Out of 19 buffaloes treated with Fertivet, 13 (68.42 %) exhibited oestrus. Medium, weak and intense heat was shown by 7 (53.8%), 5 (38.4%) and 1 (7.6%) buffaloes respectively. 8 (61.53%) buffaloes conceived to first service, while 3 (15.3%) conceived to second service with CR of 84.60%.

In Synchromate-B treated group, oestrus was induced in all (100%) buffaloes. Medium, weak and intense heat was observed in 23 (63.88%), 11 (30.60%) and 2 (6.55%) buffaloes respectively. Out of 36 buffaloes, 15 (41.66%) conceived to first, 4(11.11%) to second and 6 (16.16%) buffaloes to third service with 69.4% C.R.

Present findings regarding Fertivet therapy (68.42%) are comparable with 75% reported by Galhotra *et at,* (1971) and 73.2% by Kodagali *et al* (1981). However, lesser response was reported by Mohanty (1972) and Deopurkar (1974) with Prajana capsules and Rathore and Pattabhiraman (1977) with aloes compound treatment in cattle. The CR recorded in the present studies is higher than that in cattle (42.40%) recorded by Galhotra (1971); 50% by Mohanty (1972); 60% by Jadhao and Deshp -ande (1975); 40% by Kaikini *et al* (1980) and 50.34% by Kodagali *et al* (1981) but lower than (100%), Deopurkar (1974) and Kaikini *et al* (1977).

Induction of oestrus (100%) with SMB implant in buffaloes recorded in the present study is in full agreement with the findings of Joshi *et al* (1990) in Red Kandhari cows and Rao and Rao (1978) in buffaloes using chloprostenol.

Present finding regarding perfect synchronisation (100%) of oestrus in buffaloes is higher than that recorded (95%) by Wilt-bank and Kasson (1968); 85% by Holtz *et al* (1979); 93.8% by Kaneda *et al* (1978) and 52% by Medvedev *et al* (1981) in cows.

C.R. of 41.66% in SMB implant synchronised buffaloes in present studies is lower than that reported by Wilt-bank and Kasson (1968), and Rao and Rao (1978).

It is concluded that summer anoestrus in buffaloes can be effectively treated with Fertivet and SMB implant successfully. The induction of oestrus with SMB implant is superior, whereas C.R. at first service is higher with Fertivet therapy.

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Comparative Studies On Oestrus, Oestrus Behaviour And Oestrus Cycle In Mares Of Different Breeds Under Indian Tropical Conditions

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ABSTRACT

A study on 600 warm blooded and cold blooded mares was taken up for a period of 2 yrs. They were divided in three groups: Horse breeding, Pony breeding and Mule breeding. 50% of Horse breeding and mule breeding mares were imported from France, while pony breeding mares were of Indian origin. Significant differences were noticed in oestrus cycle, signs of oestrus, follicular activity number of follicles and pattern of ovulation between the three groups. Heavy draft purpose (Cold blooded) mares exhibited silent heat more frequently and showed more erratic reproductive behaviour. Non-ovulatory heat periods were also more pronounced in them.

* * *

Equines are polyoestrus but seasonal breeders. Breeding season is short near poles and in tropic and semitropic areas it is extended, were both ovulatory and non ovulatory oestrus can be observed almost throughout the year. They are only spontaneously ovulating species, in which oestrus without ovulation is common. Breed wise and individual variations in oestrus cycle and ovulatory process of mares have been reported (Mckenzie and Andrews, 1939; Day,

1939; Andrews and Mckenzie, 1941; Cunning, 1942; Gibbons, 1966). Marked differnces have been noticed between mares of same groups and also between successive cycles in individual animals (Bown *et al*, 1978). Silent heat or quiet ovulation has also been reported. Cooper and Wart (1975) and Neguin *et al* (1989) observed that breeding seasons can either be extended or altered by extending photoperiodicity and mares can be bred at any time of the year.

Materials And Methods

These observations on 600 mares. (300 warm blooded and 300 cold blooded) were recorded for a period of 2 years (1988- 1990). Cold blooded mares are draft purpose heavy mares while the hot blood mares are the racing type light mares having Arabian blood. They were grouped as under.

Sr. No.	Groups	Horse breeding	Mule breeding	Pony breeding	Total
1	Warm blooded	68	193	39	300
2	Cold blooded	10	276	14	300

The mares belonged to this well established institution in Northern India and were maintained under standard identical managemental conditions under semi-liberty system. Breeding season extended from February through October. Parameters considered were : (i) Oestrous cycle, (ii) Signs of oestrus, (iii) Follicular activity, (iv) Pattern of ovulation. and (v) Number of ovulations.

All the mares were teased daily by showing a teasing/testing pony for 2-3 minutes. During this period, the pony would excite the mares sexually and make love, catalysing the normal phenomenon of reproduction. Follicular acitivity was then detected during the oestrus period by gynaec examination. The length of oestrous cycle in all the mares ranged between 18-23 days. Cold blooded mares had slightly extended cycle but the differences were not significant.

Results and Discussions

(i) **Oestrus cycle** - There was a clear demarcation of all the four stages (Prooestrus, oestrus, metestrus, dioestrus) with no difference between lactating and non-lactating mares.

(a) *Proestrus* - On an average pro-oestrus was noticed for a period of 3 days as described by Seaborn, (1925). Local histo-morphological examination revealed hypertrophic vaginal mucosa, degeneration of stroma and increase in number of lymphocytes.

(b) *Oestrus* - The period of oestrus varied from 4 to 8 days. The mares became excitable, squealed, sought company of other mares or ponies (vasectomised), raised the tail and showed winking of vulva. Some warm blooded mares became aggressive. All the mares showed interest in male but most of the cold blooded mares remained averse and didn't even accept the teaser.Vaginal mucosa became pink in cold blooded mares but colour changes were not consistant in warm blooded mares which is in agreement with the observations of Roberts (1956) and Zemjanis, (1962). The ovulation was essentially at the end of oestrus but 50% cold blooded mares ovulated without showing signs of oestrus. Follicles varied from very soft to tender and 2 to 3 follicles could be palpated.

(c) *Met-oestrus* - Mucous secretion ceased and the vaginal mucosa became reddish in all the mares who did not accept the male even when kept free in paddocks. The cervix remained partially open.

(d) *Di-oestrus* - All signs of oestrus disappeared. Cervix got constricted. Vaginal m.m. turned pale. Ovaries in most mares were reduced in size. In majority of mares, specially in cold blooded, the follicles were still present on the ovaries.

(ii) **Ovulation** : Both unilateral and bilateral ovulations were observed. Follicle size at maturity varied from 15 to 50 mm. Diameters of single and double preovulatory follicles varied. Follicular activity was faster in cold blooded mares with more number of follicles developed than in warm blooded mares. Majority of ovulations in cold blooded mares were on left ovary while these were equal in both ovaries in warm blooded mares. These findings differ with those of Nishikawa (1959) but are in partial agreement with those of Osborn (1966) who observed 55.6% ovulations in left ovary. Mares went off oestrus 18 to 30 hrs post ovulation. This finding is in agreement with that of Satosh and Hoshi (1933). Size of follicle did not indicate the degree of maturity. However, ovulation was related to the consistancy and shape of follicle. Before ovulation, the follicle became soft and fluctuating as observed by Ginther and Pierson (1989). The diameter of the follicle was greater during the months of April-May than in July-August and the conception rate was also higher during April-May than in July-August. Oestrus during the months of April to June and in September was invariably ovulatory, while in remaining period ovulation was recorded only in 40-50% mares. Incidence of multiple ovulations was over 40% in all types of mares. Highest conceptions were during May/June and September. Majority of pregnancies were in opposite horns to the ovulations (cross pregnancy).

Foal Heat : It was observed in most of mares between 7 to 11 days post-partum. Follicle was palpable on 10th and 11th days. Few cold blooded mares showed mature follicle on 13-14 days. This is in partial agreement to that of Day (1939) who observed that mares came in foal heat 5-7 days after parturition and remained in heat for 5-6 days. Andrews and Mckenzie (1941) observed ovulation in 75% mares by 14th day postpartum. Trun(1950) observed 77% mares in oestrus between 7-10 day after foaling. Mcdonald (1977) observed foal heat within 2-13 days after foaling and Hafez (1974) in 5-15 days after foaling.

Salient findings of present studies are :

(1) Difference in oestrus cycle and ovulation pattern existed in foaling mares, barren mares and maiden mares but no difference was observed between lactating and non-lactating mares. Barren, undernourished and old mares had long oestrus periods.

(2) Cold blooded mares exhibited more silent heats and very erratic oestrus behaviour.

(3) Majority of mares had longer oestrus period during spring season.

(4) Few foaling mares remained in oestrus for 10-20 days and did not ovulate.

(5) Ovulation was related to the consistancy of the follicle.

(6) Foal heat was exhibited in 55 to 60% mares, with ovulation 12-13 days post-partum.

(7) Highest conception rate was during the months of May/June and September.

(8) In majority of cases the pregnancy was noticed in opposite (contra-lateral) horn from where the ovulation occurred.

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A Study On Conception Rate In Purebred Sahiwal And Jersey x Sahiwal Crossbred Cattle*

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ABSTRACT

Studies on eonception rate (CR) in Sahiwal and Jersey x Sahiwal cows and bulls revealed highly significant (P<0.01) effect of sex and parity and non-significant (P<0.01) effect of season on C.R.

* * *

The present investigation was undertaken to find out the influence of sex of calf, parity and season of insemination on conception rate (CR) both in Sahiwal and Jersey x Sahiwal crossbred cows (Sharma and Bhatnagar, 1975, Singh and Singh, 1990 and Sahota *et al*, 1990). Similarly, the C. R. of Sahiwal and Karan-Swiss bulls in terms of fertility index was also estimated.

Materials and Methods

The material for the present work comprised 147 (46 purebred Sahiwal and 101

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Jersey x Sahiwal crossbred) cows. The frozen semen belonged to 16 Sahiwal and 5 Karan-Swiss bulls of NDRI, Karnal under Associated Progeny Testing Programme at NVC Nagpur.

Standard methods were used to calculate the CR in cows and fertility index of bulls. The data was collected to study the variation due to sex of calf, parity and season of insemination from the breeding records of the college Cattle Breeding Farm, Telankhedi. Analysis of variance technique was used for statistical treatment.

Results and Discussion

Sahiwal and crossbred animals required 2.32±0.25 and 2.32±0.26 number of inseminations per conception (Table-1), being similar for both the genetic groups.

Analysis of variance indicated highly significant (P<0.01) effects due to sex and parity in Sahiwal. The number of inseminations per male calf born was more than the female in both Sahiwal and their crossbreds, although crossbreds did not show significant effect. Paritywise analysis indicated that the average number of inseminations per conception was significantly higher in first parity both in purebred and crossbred animals. The influence of seasons on number of insemination per conception in both Sahiwal and Jersey x Sahiwal crossbred females revealed non- significant (P<0.05) effects.

Singh and Kharche (1985) reported similar conception rate in crossbred females while Sharma and Bhatnagar (1975) and AICRP report on crossbreds at Rahuri (Anon, 1986) indicated higher conception rates. Singh and Singh (1990) observed non significant effect of season of insemination on conception rate. However, Sharma and Bhatnagar (1975) and Sahota *et al* (1990) reported significant effect of season on number of inseminations per conception.

Fertility index was 0.39±0.04 and 0.41±0.05 for Sahiwal and Karan-Swiss bulls. The highest fertility index was recorded by Sahiwal bull Nos 1056 (0.80) and 403 (0.66) and lowest by Nos. 494 (0.21) and 446 (0.22) Similarly, highest fertility index was recorded by Karan-Swiss bull Nos 3489 (0.56) and 3460 (0.50) and lowest by No. 3566 (0.30). The variation in fertility index amongst bulls of these two breeds may be due to the inherent differences in quality of frozen semen and its use on different females.

Table 1 : **Sex-wise and paritywise mean and standard error of number of inseminations (frozen semen) per conception in Sahiwal and JxS crossbred cows.**

Genetic Groups		Sex		Parity			Pooled over
		M	F	I	II	III	
Sahiwal cows	$\bar{X}$	2.95 [a]	1.77 [b]	3.66 [a]	1.18 [b]	1.14 [b]	2.32
	SE	0.40	0.30	0.34	0.09	0.10	0.25
	N	47	54	47	33	21	101
J x S crossbred cows	$\bar{X}$	2.72	1.92	3.33 [a]	1.14 [b]	1.13 [b]	2.32
	SE	0.28	0.24	0.23	0.06	0.10	0.19
	N	80	79	86	51	22	159

Means carring same superscript do not differ significantly from one another at P<0.05.

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Factors Affecting Number Of Inseminations Per Conception In Gir Cows*

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Number of inseminations per conception (IPC) determines the fertility status of cow. Reduction in their number minimises the cost of rearing and increases the productive life. The present investigation was therefore undertaken to study the factors affecting IPC in Gir cattle.

Material and Methods

Records of381 inseminations of Gir cows maintained at Cattle Breeding Farm, Junagadh were used for the present study. Season-Wise and parity data of 8 years (1981-88) was analysed. The data was further classified according to the 12 service sires used and analysed by fitting constants (Harvey, 1966). The linear model was used to find out the effect of period and season of calving, parity order and service sire on number of inseminations per conception. The DMR test (Kramer, 1957) was used to test the differences among the means.

Results and Discussion.

The overall average number of inseminations per conception was 1.96±0.12 in Gir cows. These results are in accordance with those (2.01) reported by Basu *et al,* (1979) in Sahiwal cows.

Differences in trait due to period of calving were non-significant, which could be attributed to less variation in environmental and managemental condtions. Kumar and Bhat (1979) reported similar results in Hariana cattle. Seasonal differences were also non significant. However, the cows that calved

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during rainy season required more inseminations (2.03±0.18) as compared to summer and winter. Similar trend with significant effect of season on this trait was reported by Basu *et el* (1979) in Hariana cows. Variations in number of IPC due to parity order were found to be highly significant (P< 0.01). Perusal of DMR test revealed that more number of IPC were required in the first than the rest of parities. These results are in agreement with those of Kumar and Bhat (1979). Service sire variations were observed to be non-significant. It might be due to similarity between sires as most of them belonged to the same herd.

The present findings support the view that heifers require more number of inseminations per conception.

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A Note On Biochemical Attributes Of Cervical Mucus In Normal And Repeat Breeding Cross-bred Cows After Synchronisation Of Estrus With PGF_2 - alpha (Dinofertin).

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Cervical mucus plays a vital role in fertility and breeding efficiency of a cow by providing an immediate nourishing protective environment to spermatozoa deposited in female genital tract. Important biochemical changes occur in cervical secretion during estrus influencing penetrability of spermatozoa through cervical mucus (CM). Information on biochemical profile of CM in normal and synchronised repeat breeders is scarce.

Material and Methods

Experimental groups comprised of nine normal and repeater cross-bred cows. Minimum one estrus of each experimental animal was studied for biochemical profile of CM, prior to synchronisation with single IM injection of PGF_2 alpha (Dinofertin 25 mg.) The CM samples were collected at mid-estrus aseptically using syringe and pipette as per Reddy (1973). These samples were then

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analysed for Calcium (Trinders, 1960), Inorganic Phosphorus (Gomori, 1942), Cholesterol (Wybenga, 1970) and total Proteins with modified Biurate method.

Results and Discussion

(a) *Calcium* (Ca): The mean Ca level (mg/100 ml) in CM of normal and repeat breeder cows was 7.10±0.612 mg. and 7.22± 0.921 mg. These levels were lower than that reported by Wani *et al* (1979) and Bora and Gupta (1988), which might be due to breed and nutritional status of animals. There was no significant difference in Ca levels of CM in normal and repeat breeder cows, during regular estrus. Similarly, after synchronisation, Ca level showed slight non-significant elevation (7.22±0.331 mg.) but reduction (6.46 ± 0.77 mg.) in repeat breeders.

(b) *Inorganic Phosphorus* : (IPmg/ 100ml) In CM of normal cows significantly higher (P<0.01) IP level (1.182 ± 0.30 mg) was noted, as compared to 0.739 ± 0.104 mg. in repeat breeders. These findings are in conformity with Wani *et al* (1979)but differ from Bora and Gupta (1988) who reported higher levels in repeater cross-bred cows.

On synchronisation too, significantly (P<0.05) higher IP level was noted in normal (1.34 ±1.12 mg.) than in repeat breeder cows (0.608 ± 0.035 mg.). However, pooled data showed no significnat difference in IP levels in regular and synchronised estrus.

(c) *Cholesterol (per 100 ml.)* : Slightly higher but non-significnat CM cholesterol level (11.83 ± 1.83 mg.) was noted in normal than (10.99±1.63 mg.) in repeat breeders . This is in accordance with Wani *et al* (1979) and Sharma and Tripathi (1985). On synchronisation also, there was no significant difference in mean CM cholesterol level of normal cows (8.06±1.10 mg.) and repeat breeders (7.84± 1.046 mg.) However, significantly higher (P<0.01) level was noted in regular estrus (11.41±00.23 mg.) than in synchronised estrus (7.95± 0.791 mg.) of both the groups.

(d) *Total Proteins (per 100 ml.)* : The average concentration of total proteins in CM of normal cows slightly higher (0.456± 0.063 mg) than in repeat breeders (0.408±0.051 mg.), but non significant. This is in agreement with Wani *et al* (1979) and Umashanker *et al* (1984). After synchronisation, total protein contents in both groups declined non-significantly which is in partial aggreement with Prasad *et al (1981).*

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Mycoplasma Infection In Repeat Breeder Bovines

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ABSTRACT

30 Samples of uterine scrappings and mucous of repeat breeder bovines were processed on mycoplasma broth media using standard techniques. On the basis of various biological, biochemical and serological tests, the isolates were typed as Mycoplasma bovis with an incidence of 7.14% in buffaloes and 6.25% in cows.

* * *

Experimental and field studies showed that a number of mycoplasma species have pathogenic potential and should be considered in any reproductive evaluation involving infertility or abortion. Mycoplasma species have been isolated from the bovine genital tract and pathogenic strains are considered as predisposing to infertility (Edward *et al*, 1947; Edward 1950). Hirth *et al* (1967) showed that M. bovis remained viable in frozen bull semen for as long as 18 months when added prior to extension and freezing in liquid nitrogen. Ten of 12 heifers inseminated with contaminated frozen semen became repeat breeders, and at necropsy four of eight had varying degrees of chronic suppurative salpingitis, chronic endometritis and ovarian adhesions.

Materials and Methods

A total of 30 samples of uterine biopsy and mucous were collected aseptically by Nielsen biopsy instrument fitted with trocar and cannula from 14 repeat breeder buffaloes and 16 cows. These were processed for the isolation of Mycoplasmatales. Various media required for isolation were prepared as per the method of Erno and Stipkovits (1973) modified by Srivastava (1982).

The uterine mucous and scrappings were inoculated in 5 ml of liquid medium and incubated at 37°C for one hour. 0.5 ml of the inoculated medium was then transferred to both liquid and solid media and incubated at 37°C for 7-10 days. The solid agar plates were incubated under 10% CO_2 tension. The inoculated liquid media were observed

Fig.1. Characteristic fried egg colonies of Mycoplasma bovis

regularly for change in pH for indications of any growth. Similarly, solid agar plates were examined for the presence of colonies under stereoscopic microscope. The isolates were typed as per the recommendations of the sub-committee on the Taxonomy of Mycoplasmatales (1979). The identification of isolates was done on the basis of biological and biochemical characters.

Results and Discussion

Out of 30 samples collected from the genital tract of repeat breeder cows & buffaloes, one sample each of cow No. 17 (cervicitis) and buffalo No.23 (endometritis) were found positive for mycoplasmatales. Both isolates showed typical morphology of the organism and produced characteristic fried egg colonies (Fig.1) on solid mycoplasma media, which failed to revert back to bacterial growth in the absence of penicillin and thallium acetate and were sensitive to dignitonin. In the primary isolate there was not much change in the pH of liquid media, however, characteristic colonies were seen on solid media. Sample number 17 of cow showed slower growth than the sample number 23 of buffalo. These were classified under the genus mycoplasma. These strains when subjected to biochemical tests were found positive for Film and spot formation and phosphatase production. The other biochemical tests: Glucose fermentation, arginine catabolism, urea hydrolysis and serum digestion were found negative. In growth inhibition test, both the isolates produced 3 to 5mm growth inhibition zone around the M. bovis antisera disc, on the basis of various biological, biochemical and serological tests both the isolates were typed as Mycoplasma bovis.

The results revealed an incidence of 7.14% mycoplasma infection in buffaloes and 6.25% in cows with an overall incidence of 6.69% in repeat breeder bovines. Similar results were reported in infertile cows by Witkowski *et al* (1984) who found 6% incidence of mycoplasma infection. An incidence of 1.5 to 8.96% mycoplasma infection in buffaloes with reproductive problems is reported from India (Pal *et al*, 1982; Katoch *et al*, 1982).

In the present study the two positive cases were having cervicitis and endometritis. These observations are comparable with the findings of Hartmann *et al* (1964) who produced endometritis, salpingitis and salpingo-peritonitis in 7 out of 8 virgin heifers inoculated with mycoplasma. Hirth *et al* (1967) showed that M. bovis remained viable in frozen bull semen. By using such contaminted semen, 10 out of 12 heifers became repeat breeders and on necropsy, varying degrees of chronic suppurative salpingitis, chronic endometritis and ovarian adhesions were noticed. These studies clearly indicate the virulence and importance of mycoplasma in repeat breeding condition.

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Quantitative and Qualitative Studies On The Genital Bacteria Of Buffaloes*

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Cervical mucus samples were examined from 35 normal fertile and 65 repeat breeder Surti buffaloes. Bacterial load was 1.475 x 10^3 per ml. in normal and 38.05 x 103 per ml. in repeat breeder buffaloes. The average bacterial load was about 26 times higher in repeat breeder than in normal animals. 73 samples were culturally positive for bacteria, of which 18 were from normal and 55 from repeat breeders. Of the 97 isolates recovered, gram positive bacteria (81.84%) predominated over gram negative (18.16%) bacteria. 38 isolates were diptheroids (39.18%); 25, gram positive cocci (25.77%); 16, gram positive spore forming bacilli (16.48%) and 18 gram negative bacilli (18.56%).

* * *

Infectious infertility plays a major role and attributes to more than one third of the total infertility (Kodagali *et al*, 1973). It is also evident from the available literature, that there is no remarkable difference in the types of organisms and frequency of occurrence of organisms in the genital tract of Surti buffaloes under different reproductive status. It is, therefore, speculated that there may be quantitative difference of organisms in the genital tract of animals having reproductive disorders and normal animals. Keeping this in view, present study was undertaken.

Materials and Methods

Cervical mucus samples were collected aseptically from 100 (35 normal, 65 repeater) buffaloes by rectovaginal technique (Panangala *et al*, 1978).

Quantitative study : Equal quantity of 1% amyl acetate solution was added to each sample and then kept at room temp. for 45mts. with frequent shaking to dissolve the mucus. The bacterial load was estimated by Standard Plate Count (SPC) method, which was done by mixing SPC agar medium with tenfold dilution of mucus from 1:10 to 1:1000, depending upon the expected bacterial load. Two SPC plates (100x17 mm with 20 ml of SPC agar) were prepared from each dilution and incubated at 37°C for 48 hrs. The colonies were counted using colony counter. Total bacterial load per ml of mucus was calculated from colony count of these two SPC plates.

* Part of M.V.Sc. thesis submitted by the first author.
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Qualitative study : Isolation of the bacteria was done as per Cruickshank *et al* (1975). Each of the isolate was further identified into various genera and spp. as per Cowan and Steel (1970).

Results and Discussion

Quantitative study : The bacterial count ranged from 0 to 10,600 (Av. 1.475×10^3) per ml of mucus in normal fertile animals, whereas, in repeat breeders it varied from 290 to 1,37,000 (Av. 38.05×10^3). The count was about 26 times higher in repeat breeders. Higher count in repeat breeder cows was reported by Panangala *et al* (1978). Raghavan *et al* (1971) opined that when the bacteria normally present in the genital tract increase in number, it may lead to repeat breeding.

Qualitative study : Out of 35 samples, 17 (48.38%) in control group and 10 out of 65 (15.38%) samples in repeat breeding group were sterile. Gunter *et al* (1955) reported 33% sterile samples from control animals. Dholakia *et al* (1987) reported 32.08% sterile samples in repeat breeders. The study clearly indicated that the frequency of occurrence of organisma was higher in repeat breeders than in normal group. The number of samples yielding 1,2,3 and 4 isolates were 15,3,0 and 0 for normal buffaloes, and 39, 12, 3 and 1 for repeat breeders respectively. Shah and Dholakia (1983) also isolated upto 4 types of organisms from buffaloes. The proportion of sterile, single and multiple isolates remained significantly different in both the groups. 18 of the 35 samples from normal animals, yielded 21 culturally positive isolates, Whereas 55 of the 65 repeat breeder yielded 76 positive isolates. The organisms isolated from normal animals were mostly those which were of normal flora (commonsal) of female genital tract. Krishnamurthy *et al* (1974) opined that

Table 1 : **Frequency of occurrence of different species of bacteria in cervical mucus of normal fertile and repeat breeding buffaloes**

Sr.No.	Name of isolates	Normal group		Repeat breeder group	
		No.	%	No.	%
1	*Staphylococcus aureus*	1	4.76	7	9.22
2	*Staph. epidermidis*	3	14.28	4	5.28
3	*Streptococcus faecalis*	-	-	1	1.31
4	*Str. bovis*	-	-	1	1.31
5	Micrococcus	3	14.28	5	6.58
6	*Corynebacterium pyogenes*	2	9.53	17	22.38
7	*Coryn. ulcerans*	1	4.76	2	2.63
8	*Coryn. bovis*	1	4.76	4	5.28
9	*Coryn. murium*	1	4.76	3	3.95
10	*Coryn. xerosis*	-	-	3	3.95
11	*Coryn. equi*	-	-	1	1.31
12	*Kurthia spp.*	-	-	2	2.63
13	*Listeria monocytogenes*	1	4.76	-	-
14	*Bacillus circulans*	-	-	3	3.95
15	*B. macerans*	1	4.76	1	1.31
16	*B. badius*	-	-	2	2.63
17	*B. cereus*	-	-	3	3.95
18	*B. megaterium*	-	-	1	1.31
19	*B. firmus*	-	-	2	2.63
20	*B. coagulans*	-	-	2	2.63
21	Untypable *Bacillus spp.*	-	-	1	1.31
22	*Escherichia coli*	4	19.06	5	6.58
23	*Enterobacter cloacae*	-	-	1	1.31
24	*Pasteurella haemolytica*	1	4.76	1	1.31
25	*Acinetobacter lwoffii*	2	9.53	-	-
26	*Klebsiella aerogenes*	-	-	1	1.31
27	*Pseudomonas aeruginosa*	-	-	1	1.31
28	Unidentified gram negative bacilli	-	-	2	2.63

bacteria normally present in the genital tract may become pathogenic under certain conditions resulting in repeat breeding. Altogether 27 spp. of bacteria were isolated (Table1). The types of bacteria were mostly common in both the groups. Shah and Dholakia (1983) also obtained similar findings. The spp. like *Staphylococcus aureus, Corynebacterium pyogenus* and *Pseudomonas aeruginosa* appeared in higher frequency in repeat breeders, which was significant as they have been incriminated as potential pathogens rendering the female genital tract more harmful for sperm viability.

Out of the total 97 isolates, gram+cocci were 25 (25.78%); gram+ nonspore forming bacilli 38 (39.18%); gram spore forming bacilli 10 (16.48%) and gram negative bacilli 18 (18.56%) indicating dominance of gram positive bacteria. This confirms the findings of Dholakia *et al* (1987). In contrast, predominance of gram negative bacteria was observed by Deka *et al* (1979). Diphtheroids formed predominant group (36.08%) of all the isolates, which is in accordance with Shah and Dholakia (1983), who reported 31.57% incidence of diphtheroids.

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Progesterone, Oestradiol 17-B, Triidothyronine And Thryoxine Profile In Cyclic Berari (Nagpuri) Buffaloes

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ABSTRACT

The hormonal profile of progesterone (P_4), Oestradiol 17-B(E_2), Triidothyronine (T_3) and Thyroxine (T_4) was assayed by RIA technique in various phases of oestrus cycle in cyclic Berari Buffaloes.

The mean levels of these hormones on day of oestrus and at mid luteal phase were P_4 O.15± 0.13 ng/ml. 2.68±0.75ng/ml.; E_2 41.02 ± 11.11 pg/ml.; 14.98 ± 1.74 pg/ml.; T_3 1.45 ± 0.62 ng/ml.; 1.46 ± 0.59 ng/ml.; and T_4 2.88 ± 1.53; 6.36 ± 4.02 ng/ml.

P_4 level was lowest (0.15 ± 0.13 ng/ml) on the day of oestrus with maximum increase between 12th and 15th day of oestrus cycle (2.68 ± 0.78 ng/ml and 2.61 ± ng/ml). E_2 level was highest on day of oestrus (41.02 ± 11.11 pg/ml) and fluctuated between 15.84 ± 2.15 pg/ml on day 15 and 13.58 ± 1.53 pg/ml on day 23. T_3 level was higher (1.45 ± 0.62 ng/ml) on day of oestrus. T_4 level at oestrus was 2.85 ± 1.53 ng/ml. It was maximum (6.36 ± 4.02 ng/ml) on 12th day with a declining trend.

* * *

Berari buffaloes play a vital role in the livestock economy of Vidarbha. Complexities in reproduction necessiate investigation of hormonal profile in buffaloes. Studies on Progesterone, Oestradial 17-B, Triidothyronine and Thyroxine profile in cyclic Berari buffaloes were therefore undertaken.

Materials and Methods

Normal cycling Berari buffaloes of Livestock Instructional Farm, Akola were studied. The buffaloes were maintained under uniform managerial conditions and heat detection programme carried out thrice daily at 6 A.M., 1.P.M. and 6 P.M. by parading vasectomised buffalo bull. On detecting the buffalo in heat she was subjected to gynaeco-clinical examination to confirm the gonado-genital status.

Blood from jugular vein was collected on day O, 4, 9, 12, 15, 23 and 27 to estimate serum Progasterone (P_4), Oestradiol 17-B (E_2), Triidothyronine (T_3) and Thyroxine (T_4) profile by RIA method. Serum was preserved by adding 1% merthiolate solution (2-3 drops in 5 ml serum) and kept at -20°C in the deep freeze. Radio Immuno Assay was carried out for P_4 and E_2 by using coat and count kits (M/s. Ezra Company, Bombay) and T_3 and T_4 by using kits from BARC, Bombay.

Results and Discussion

1. *Serum Progesterone* (P_4) : The pattern of P_4 level in normal cyclic buffalo was lowest (0.15 ± 0.13 ng/ml) on the day of oestrus. It increased through day 4 and day 9 to reach 2.68 ± 0.75 ng/ml on day 12 (Table 1). These

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findings are in agreement with Hafs and Armstrong (1968) who reported very low P_4 levels on the day of oestrus, but statistically higher levels from day 3 to 6 in cows. The increase in P_4 concentration was maximum between day 12 and day 15 (2.68 ± 0.78 ng/ml, 2.61 ± 1.13 ng/ml) in the present study.

Gomes *et al* (1963) reported highest concentration of P_4 on day 13 after ovulation. Bachlaus *et al* (1979) reported similar observation of a minimum progesterone level (0.16 ± 0.02 ng/ml) on the day of oestrus, which rose steeply and attained a peak on day 15. However, P_4 level observed in the present study (2.61 ± 1.13 ng/ml) on day 15 is less than (5.21 ± 0.05 ng/ml) report of Bachlaus *et al* (1979). Subsequent gradual decrease was observed on day 23 and day 27 in the present study. These findings corroborate with those of Batra *et al* (1981) and Pahwa and Pandey (1983).

2. *Serum Oestradiol 17-B* (E_2) : The highest mean Oestradiol 17-B concentration of 41.02 ± 11.11 pg/ml was observed on day O, which subsequently decreased on day 4,9 and 12 to 21.02 ± 3.93, 17.18 ± 3.55 and 14.98 ± 1.74 pg/ml respectively. Further, E_2 levels fluctuated between 15.84 ± 2.15 pg/ml on day 15 to a decrease of 13.58 ± 1.53 pg/ml on day 23.

Arora and Pandey (1982) reported E_2 concentration of 19.32 ± 3.73 pg/ml on day of oestrus which declined to 5.73 ± 3.68 pg/ml on day 2 of the cycle. Further, a minor rise on day 4 and more sustained level on day 10 is reported by Bachlaus *et al* (1979). A gradual increase to a peak concentration of 31.34 ± 1.70 pg/ml before oestrus is reported by Arora and Pandey (1982). Thus the pattern of E_2 concentration observed in present study is in close agreement with Dobson and Dean (1974), Batra *et al* (1980) and Arora and Pandey (1982). However, it is higher than that reported by these workers but lower than the report of Kampanputna *et al* (1979). This profile is comparable with the development of follicles throughout the late luteal phase and probably also reflects the short term fluctuations in the secretions of steroids from the same follicles (Baird, 1978). Jain and Pandey (1983) reported an increase in the secretion of E_2 within hours of each pulse of LH indicating that the follicles in buffaloes are extremely sensitive to comparatively minor fluctuations in LH concentration and reflected by a positive correlation between LH and E_2. The subsequent minor peaks noted on day 15, 17 and 19 in the present study were similar to Batson *et al* (1973).

3. *Triidothyronine* (T_3) : On the day of oestrus the mean level of T_3 was 1.45 ± 0.62 ng/ml. It showed slight declining trend on day 4 to 1.05 ± 0.66 ng/ml and remained constant (1.46 ± 0.59, 1.46 ± 0.35 ng/ml) in early luteal (day 9) and midluteal (day 12) phase. Then it showed declining trend except on day 23.

Galhotra *et al* (1988) reported variations in T_3 values during the various phases of production status. Likewise, in the present study, T_3 level also fluctuated without any definite trend during normal oestrus cycle. The T_3 and T_4 concentrations varied between different phases of oestrus cycle, with a relatively higher level on day of oestrus.

4. *Thyroxine* (T_4) : Thyroid hormones maintain basal body metabolism. Thyroid dysfunction can disturb several body functions including reproduction.

The average T_4 level on day of oestrus was 2.88 ± 1.53 ng/ml. It decreased on day 4 to 1.17 ± 0.55 ng/ml and thereafter showed an increasing trend to reach peak level of 6.36

± 4.02 ng/ml on day 12, with subsequent declining trend. The values of T_4 estimated under the present study in Berari buffaloes are comparatively very low than those recorded by Deshmukh *et al* (1988) in Murrah buffaloes (26.3 to 32.6 ng/ml) after parturition till 150 days under different levels of feeding.

The low values of T_4 in present investigations may be due to lactating buffaloes. This finding is in agreement with Galhotra *et al* (1988) who recorded mean T_4 levels of high, medium and low yielders ranging from 16.9 to 50.00, 14.7 to 39.60 and 23.5 to 47.12 ng/ml respectively.

Higher values of T_3 and T_4 recorded during oestrus are similar to those reported by Abdo *et al* (1969) in the camel; Sharma and Sharma (1976) and Agarwal *et al* (1985) in cycling goat. Vadodaria *et al* (1978) reported maximum PBI activity in Surti buffaloes at the beginning of heat which fell to a level observed close to ovulation. A gradual rise

Table 1: **Mean levels of progesterone, oestradiol 17-B, Thyroxine and Triidothyronine during oestrous cycle in normal cycling Berari buffaloes.**

Sr. No.	Hormonal levels	n=6	Day 0	Day 4	Day 9	Day 12	Day 15	Day 23	Day 27
1	Progesterone (ng/ml)	Range Av. SE	0.10 – 0.23 0.15 ± 0.13	0.10–1.00 0.64 ± 0.16	1.60–4.20 1.60 ± 0.70	0.60–4.80 2.68 ± 0.75	0.6–4.80 2.61 ± 1 .13	0.10–5.40 1.68 ± 0.194	0.10–4.20 1.81 ± 0.567
2	Oestradiol 17-B pg/ml	Range Av. SE	15.98–82.40 41.02 ± 11.11	8.59–50.12 21.02 ± 3.93	6.44–31.37 17.18 ± 3.55	10.68–21.04 14.98 ± 1.74	10.98–26.25 15.84 ± 2.15	9.19–19.44 13.58 ± 1.53	9.87–20.34 14.90 ± 1.69
3	Thyroxine ng/ml	Range Av. SE	1.05–7.88 2.88 ± 1.53	0.55–2.57 1.71 ± 0.55	0.82–4.79 2.34 ± 1.04	0.84–26.98 6.36 ± 4.02	0.78–4.22 2.11 ± 0.85	0.48–3.65 2.35 ± 0.82	0.65–4.74 2.43 ± 0.90
4	Triidothy-ronine ng/ml	Range Av. SE	0.72–2.69 1.45 ± 0.62	0.43–2 .07 1.05 ± 0.66	0.37–2.27 1.46 ± 0.59	1.04–2.16 1.46 ± 0.35	0.66–1.89 1.26 ± 0.41	0.65–2.85 1.74 ± 0.61	0.65–2.05 1.48 ± 0.44

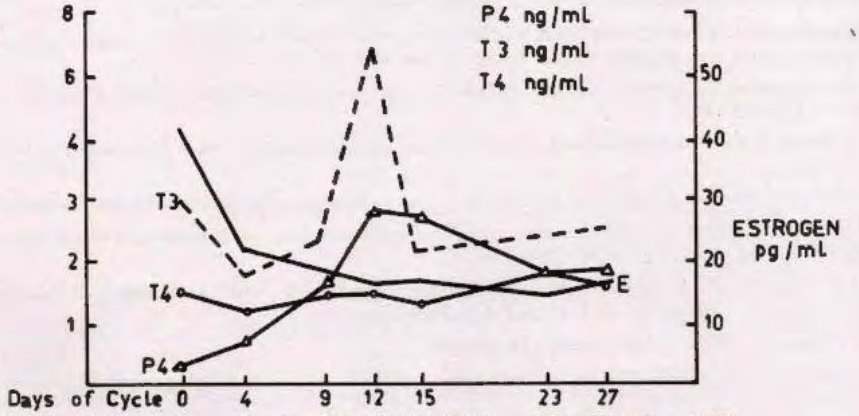

Fig. 1-ESTRADIOL 17-B, PROGESTERONE, THYROXINE AND TRIIODOTHYROXINE PROFILE IN NORMAL CYCLING (CONTROL) BUFFALOES.

during luteal phase with elevation at the beginning of heat is also reported by them. Jindal *et al* (1988) reported high plasma T_3 levels during oestrus phase in buffaloes. However, the plasma T_4 concentration did not change much during luteal phase. Present observations are in agreement with these workers. The higher T_3 and T_4 values during oestrus may possibly be associated with increased estrogenic activity during these periods of oestrous cycle (Ingbar and Wober, 1974). Soliman and Reinkee (1954) and Feldman (1956) also suggested that increase in thyroid activity at oestrus in ewes may be due to increase in oestrogen level or due to direct release of TSH from pituitary along with release of FSH and LH. Thus the T_3 and T_4 levels recorded in the present study are parallel to the reports of these earlier workers.

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Efficacy Of Prostaglandin F_2 Alpha Analogue (Dinofertin) For Synchornization Of Estrus In Subestrous Cows.

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ABSTRACT

Dinofertin (25 mg) treatment (1M) was given for inducing estrus in 12 suboestrus Red Kandhari cows. Six cows(50%) exhibited intense heat 3.66 days (Av) following treatment. None of the untreated (control) cows came in heat.

Materials and Methods

Twenty-four Red Kandhari cows belonging to RK unit, MAU Parbhani, with palpable CL/ovarian activity but not exhibiting external signs of heat (>90days) were selected for study.Half of the cows (12) were given single dose 5ml IM inj. Dinofertin (25mg of PGF_2 alpha analogue) and the remaining (12) cows kept as control. Both the treatment and control group cows were gynaeco-clinically examined at uniform intervals for noting ovarian changes. Visual observations and parading of Teaser bull for heat detection was carried out.

Cows exhibiting heat were inseminated and they were followed up for pregnancy or subsequent oestrus.

Results

Corpora lutea were found regressed on day 2 to 5 following Dinofertin treatment with 100% efficacy. Out of 12, six cows (50%) exhibited heat following regression of CL within 5days (120 hrs) of treatment. Six cows that failed to exhibit heat after regression of CL were followed for 21 days post treatment. All the cows of control group did not exhibit oestrus.

Animals which exhibited heat were inseminated. Pregnancy was confirmed in 4 out of the 6 inseminated cows with conception rate (CR) of 66.4%.

Discussion

The observations regarding response to the therapy (50%) are in agreement with those of Eddy (1977) and Dorowin and Selin(1989), However, lower response(20%) was reported by Ott *et al* (1980). Higher response (80%) was reported by Becze *et al* (1980) with75% CR and Thakur *et al*(1989) who reported 92.31% response. Thus it can be inferred that administration of 5ml inj. Dinofertin (25mg PGF_2 alpha analogue) can give 50% response in silent oestrus cases with 66.4% CR .

The differences in response and also in conception rate may be due to variations in breeds, plane of nutrition, environmental factors, managerial practices and hormonal profiles of the animals studied.

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Fetotomy In Dystocias Due To Monstrosities In Bovines

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ABSTRACT

Twenty three cases of foetal dystocia were successfully treated by performing fetotomy with Thygeson's fetotome. These conditions were : Schistosomus reflexus, Perosomus elumbis, Dicephalus, Dipygus, Thoracopagus, Bull dog calves, Hydorcephalus, muscular hypertrophy and subcutasneous lymphatic cysts. The number of cuts to deliver the fetus per-vaginum varied form 1 to 3 without any post-operative complications.The dam survival rate was 88.6% in buffaloes and 100% in cows.

* * *

Fetotomy is the reduction in the size of fetus by removal or destruction of its parts in order to permit delivery and is indicated where forced traction and mutation fails to extract the dead foetus. Percutaneous fetotomy in handling dystocias due to monstrosities described by Bierschwal and deBois(1972) as practiced in cattle and buffaloes is presented in this paper.

Materials and Methods

Twenty three cases of dystocias due to monstrosities in buffaloes(n=15) and cross bred cows(n=8) were handled in specialized unit of Veterinary Clinics during a period of 3 years (1986-89). Perenial desensitization was achieved by epidural anaesthesia and the fetus was properly lubricated using parachlorgel. Before resorting to percutaneous fetotomy using Thygesen's fetotome, monsterosity was diagnosed which was confirmed on extraction of the fetus.

Results and Discussion

Anomalies of Head and Neck : In hydrocephalic fetuses (Buffalo 2 and Cow 1) the fluid was evacuated by incising the overlying skin. In one buffalo fetus, the cut was made at the atlanto-occipital junction and both the divergent cranial bones removed while in other two fetuses, the parietal bones were bisected to reduce the size of the head, as per Bierschwal and deBois (1972). Since the

cow fetus was associated with contorted joints, it required two more transverse bisections at mid thorax and at lumbar region. Of the two bull dog calves encountered in buffaloes, one required amputation of head at atlanto-occi pital junction only, while in the other transverse bisection of lumber region and longitudinal bisection of pelvis had to be performed to extract the emphysematous fetus. Foetus with muscular hypertrophy of neck required amputation at base of neck.

Anomalies of Trunk : Three schistosomus reflexus fetuses were delivered by percutaneous fetotomy. Buffalo fetus was extracted by giving 2 cuts, one involving removal of a forelimb and the second involving bisection of fetal trunk at the point of spinal angulation. Both the schistosomus reflexus fetuses in cattle were in transverse presentation. In one case, the fetus was delivered with single cut made at the junction of spinal angulation as per the technique of Bierschwal & deBois (1972). The second case of schistosomus reflexus, however, required 3 cuts first involving removal of head & neck, one forelimb and abdominal viscera; the second involved removal of one forelimb and thoracic cavity, and the third involved the bisection of pelvis. Perosomus elumbis fetus in cattle was in posterior presentation with hock flexion posture. Both the contracted hock joints were amputated to effect delivery. In handling muscular hypertrophy of fetal hind quarter, two cuts were made, one at lumbar region for removal of cranial part and second involving pelvic bisection to remove the fetus.

Dystocia due to subcutaneous lymphatic cysts,in the lumbar region and base of neck of fetus was relieved by giving 2 cuts at the site of cysts. First cut involved the removal of hind quarter followed by evisceration,with the second cut at base of neck resulting in removal of thoracic cavity and both the fore limbs.

Fetal dropsical conditions included fetal ascites and excessive accumulation of fluid in the abomasum in buffaloes and excessive accumulation of fluid in gastro-intestinal tract in cattle. In all such cases the abdominal wall was incised using fetotomy knife for reduction by evacuation of fluid or evisceration as per Sloss & Dufty(1980)

Embryonic duplications : The malformations characterized by duplication of fetal parts in which fetotomy was carried out successfully were diplopagus sternopagus (n=2), dicephalus dipus dibrachius (n = 3) and monocephalus tetrapus tetrabrachius (n = 1) in buffaloes and dicephalus dipus tetrabrachius (n = 1) and diprospus triophthalmic (n = 1) in cattle. Both the diplopagus sternopagus fetuses were taken out with single cut. In one monster the amputation was made in the lumbar region of the posteriorly presented component of the conjoined twin. The remaining fetus with double cranial components and single hind quarter was taken out as a single mass. The second diplopagus sternopagus fetus presented anteriorly, was cut at the junction of fused thoracic cavities. Each nearly complete component was taken out one by one.

Fetotomy of monocephalus tetrapus tetrabrachius fetus was carried out using three cuts: First at hipjoint to remove one hind limb; second at lumbar region to remove the remaining hind quarter followed by evisceration and the third at lumbar region of the second component to remove the hind quarter. The remaining cranial portion having one head, 4 fore-limbs and throacic cavity was taken out with judicious traction. Sloss and Dufty (1980) feel that fetotomy in cases of double monster is a difficult, often formidable,

task. However, Frerking (1964) suggested the technique in handling double monsters by giving six cuts. Of the three dicephalus dipus dibrachius monsters, two required only one cut involving the removal of one head while the third one required two cuts. First cut involved the removal of one head and neck whereas the other fore-limb were taken out with second cut. Presented head & neck, 2 fore-limbs and thoracic cavity were amputated with single cut in dicephalus dipus tetrabrachius fetus of cattle, followed by abdominal evisceration. Remaining nearly complete component was taken out by manipulation. Diprospus triopthalmic fetus was delivered manually in cattle following amputation at atlanto-occipital junction to remove the head with two faces.

In the present study although no definite technique of fetotomy was employed but depending on the foetal parts presented in the passage and working space in the uterus, the cuts were made in such a way that traction points are not lost, with easy extraction of amputated parts and minimum involvement of bones.

Overall survival rate of dam in relation to different monstrosities in cattle and buffaloes was 100% and 88.6%, respectively. Only two buffaloes having fetal embryonic duplication died probably owing to the development of emphysema. As per Roberts (1971), the duration of dystocia before intervention affected its outcome. Therefore, high survival rate without post-operative complications in the present study suggested that percutaneous fetotomy in dystocias due to fetal monstrosities can be performed with advantage.

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Treatment Of Summer Anoestrus In Buffaloes With Bromocriptine

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The majority of buffaloes undergo anoestrus conditions during peak summer months of the year. The ovaries are in quiescence stage with no behavioural symptoms of oestrus. Attempts have been made by various workers to treat buffaloes during summer season, with hormonal therapy with variable results (Singh et al, 1983, 1988; Chede, 1992). Singh and Madan (1989) indicated that this condition in buffaloes is possibly due to hyperprolactinaemia. In the present study, oestrus was induced with bromocriptine therapy during summer months in anoestrus buffaloes.

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Materials and Methods

Twenty randomly selected adult (6-10yrs) buffaloes in the adopted villages of ambulatory Clinics during May and June, (Max Temp. 41.2°C & Min. 22.2°C) constitute the study. All the buffaloes were high yielders and were in true anoestrus condition. The gynaecoclinical examination of these animals revealed smooth inactive ovaries without follicle or corpus luteum on it. The animals were divided into two groups. Group I of 4 animals as control which did not receive any treatment. Group II consisted of 16 buffaloes which received antiprolactin drug Bromocriptine mesylate (Chemech Laboratories Ltd, Madras) four tablets (10 mg) orally by mixing with feed for 5 days. All the 16 animals were then observed carefully daily morning and evening for behavioural signs of oestrus and finally confirmed gynaecoclinically for oestrus. The animals showing induced oestrus were inseminated with fertile buffalo semen/mated and pregnancy confirmed two months later.

Results and Discussion

The animals of group I did not show any development on ovary nor oestrus symptoms during the study. Out of 16 animals of group II, 9 (56.25%) were detected in oestrus within 8 to 21 days (Av. 13.77 days) of treatment. Two animals which showed oestrus between 18th to 21st day post treatment showed weak oestrus and did not conceive. Four animals conceived with first insemination, two animals with second insemination and one with third insemination. Pregnancy was confirmed 60 days after insemination by gynaec examination. The buffaloes which did not conceive on Ist insemination after induction of oestrus showed normal cyclicity till conception. Singh and Madan (1989) reported a significant increase in prolactin level in lactating anoestrus buffaloes during summer season. The present study supports the views of Singh & Madan (1989) by inducing oestrus with anti-prolactin drug Bromocriptine therapy with desired fertility (63.63%). Buffaloes that did not respond, may be having higher levels of prolactin in blood during summer months and the dose of Bromocriptine (10 mg) given to them might not be sufficient to suppress the increased level of prolactin.

The quiescent ovaries in seven buffaloes even after Bromocriptine administration indicate that some unidentified factors also play a role in summer anoestrus in buffaloes.

Present results indicate that hyperprolactinaemia in summer months is a major cause of anoestrus condition in buffaloes. Keeping prolactin level under control can possibly overcome this problem.

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Studies On Superovulation And Recovery* Of Embryos In Goats Treated As Donors

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ABSTRACT

Out of 12, 8 (66.67%) donor goats exhibited oestrus 59.87± 2.33 hrs. following Inj. Folligon treatment and the overall duration of oestrus was 22.5±0.23 hrs. The overall number of intact follicles was 6.62± 1.34 and corpora lutea 3.12±0.74. The overall no of ovulations, no of eggs recovered and no. of eggs fertilized was 3.12, 0.875 and 0.428 respectively. In all 7 eggs were recovered: two morula stage; one of two celled stage and four unfertilized.

* * *

Superovulation is the process by which the ovaries are stimulated to produce more than the normal number of ovulatory follicles and release more ova to exploit the superior genetic potential of an outstanding doe for 'Embryo Transfer'. For superovulation, PMSG (Pregnant mares serum gonadotrophin), FSH (Follicle stimulating hormone), HAP (Horse anterior pituitary extract) or HMG (Human menopausal gonadotrophin) can be used. But since the circulatory half life of FSH, HAP and HMG is short, repeated injections are necessary and as FSH is not easily available in India, PMSG is used. The present study was undertaken to assess the efficacy of Inj. Folligon (PMSG) for superovulation and to standardise the process of surgical embryo recovery in does.

Materials and Methods

A total of 12 Osmanabadi does and their crosses with Alpine, Beetal and Saanen aged 3 to 4 and weighing 22 to 38 kg were selected. All these dose were healthy, normal cyclic with good reproductive efficiency, earlier 2-3 normal kiddings with last kidding at least 2 months earlier. They were kept under identical feeding and managemental practices and divided into 4 groups comprising 3,3,4 and 2 does in I,II,III & IV group respectively. For synchronization of oestrus, each doe was given IM 0.5 ml (12.5 mgPG) Inj. Lutocycline (Hindustan CIBA-GEIGY Ltd, Bombay) per day for 17,16,15 and 14 days in group I,II,III and IV respectively. Superovulation was achieved by injecting Folligon (Intervet International B.V. Boxmeer, Holland) 1000 IU subcut on penultimate day of PG treatment and Inj. Chorulon (Intervet International B.V. Boxmeer, Holland) 1500 IU (HCG) I.V. 48 hours after Folligon treatment in Group I does. Inj. Folligon 1000 IU was given subcut on penultimate day of PG treatment and Inj. Chorulon 1500 IU administered I.V. 24 hours after Folligon treatment in does of Group II.

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Inj. Folligon 1000 IU was administered S.C. a day after last PG dose and Inj. Chorulon 1500 IU was administered I.V., immediately after exhibition of oestrus in does of Group III. Group IV does were treated with Inj. Folligon 1000 IU subcut a day after last PG dose and I.V. Inj. Chorulon 1500 IU 24 hrs after exhibition of oestrus.

The does were closely observed for oestrus by parading a vasectomised buck daily at 7 a.m. and 4 p.m. and also by visual observations. The does which exhibited oestrus were hand mated by offering maximum natural services. Only the does which exhibited oestrus were operated for mid-ventral laparotomy under sedation with Inj. Siquil. 1 mg per kg body weight (Sarabhai Chemicals, Baroda) and local infiltration of the site with 2% Lignocaine Hydrochloride (BAIF Laboratories, Pune) 4 to 5 days after exhibition of oestrus. Dulbeccos Phosphate Buffered Saline (DPBS) enriched with 15% goat serum and pH adjusted to 7.2 was used for flushing. After opening the abdominal cavity, genitalia was exteriorised and ovaries observed for the presence of corpora lutea and intact follicles. The uterine and oviductal embryos were recovered by flushing the uterus and oviduct simultaneously. The flushed fluid was searched for the embryos using sterioscopic microscope, at 40 x magnification.

Results and Discussion

Out of 3 does from Group I, 1(33.34%) doe exhibited oestrus after 82 hrs of Folligon treatment, with 24 hrs. duration. In Group II, 2 (66.67%) out of 3 does exhibited oestrus after 78.06±6 hrs of Folligon treatment, with 24 hrs duration. In Group III, 3 (75%) out of 4 does exhibited oestrus after 37.67±5.73 hrs of Folligon treatment, with duration of 21.33±1.33 hrs. In Group IV, 2 (100%) does exhibited oestrus after 64.8hrs. of Folligon treatment, with duration of 22±2 hrs. The exhibition of oestrus was 66.67% (8 out of 12) and time required for exhibition of oestrus was 59.87± 2.33 hrs after PMSG treatment, with 22.5±0.23 hrs duration of oestrus.

The average no. of intact follicles was 2,11.5±0.5, 5.34± 1.12 and 6.0±3.0 whereas the average no of corpora-lutea were 2,1.5±0.5, 4±0.57 and 4.0±3.0 in does of Group I,II,III and IV respectively. The no. of intact follicles was 3.25± 0.70 and 3.37±0.70. while the no. of corpora-lutea was 2.16± 0.40 and 1.57±0.29 in right and left ovary respectively. The overall eggs recovery was 24.17% and fertilized eggs was 45.83%. In all 7 eggs were recovered, out of which two morula stage, one two celled stage and four eggs were unfertilized.

The duration between exhibition of oestrus and embryo recovery was 96, 101±5, 107.34±6.9 and 96 hrs in the four goups with an overall duration of 101.5±3.06 hours.

The present finding regarding percentage of does exhibiting oestrus is lower than that of Ahmed and Maurya (1981); Tervit *et al* (1983); Agrawal (1986) and Indramani and Vadnere (1989), who reported 100, 97.14 and 100% respectively. The time required for exhibition of oestrus is higher than the findings of Ahmed and Maurya (1981) and Indramani and Vadnere (1989) who reported it as 1.2 days. However, it is in agreement with Patil *et al,* (1984) who reported it as 51.02±4.66 hours but lower than the report (4 days) of Agrawal (1986).

The finding regarding number of intact follicles is higher than 2.5 and 3.25 reported by Tervit *et al.* (1983) and Indramani and Vadnere (1989). As per Armstrong *et al* (1983) the incidence of large follicles that failed to

ovulate was higher in PMSG treated Angora goats.

The finding of ovulation to total ovarian activity (%) is lower than 49.01% reported by Patil *et al* (1984) and 76% (Indramani and Vadnere, 1989)

The finding of ovulation rate is higher than 2.8±1.6 reported by Armstrong *et al* (1982) and lower than the findings of Ahmed and Maurya (1981), Armstrong *et al* (1982), Tervit *et al* (1983), Agrawal (1986) and Rajkhowa *et al* (1989), who reported it as 12.54, 10.5±1.5, 8.8, 7.5 and 14 respectively. The lower ovulation rate may be due to differences in timings for administration of hCG in different groups of does. The other reasons are hormonal profile, nutritional status and breed differences.

The finding regarding per cent egg recovery rate is lower than that of Ahmed and Maurya (1981), Tervit *et al* (1983) and Patil *et al* (1984) who reported it as 77.41, 82 and 100% respectively. The finding regarding mean egg recovery rate is lower than the findings of Armstrong *et al* (1983), Tervit *et al* (1985), Kiessling *et al* (1986) and Rajkhowa *et al* (1989) who reported it as 7.9,6.0, 2.0 and 9.0 respectively. The present finding regarding % egg fertilization rate is lower than the findings of Ahmed and Mayrya (1981), Tervit *et al* (1983) and Patil *et al* (1984) who reported it as 77.41,82 and 100% respectively. The finding regarding mean egg recovery rate is lower than the findings of Armstrong *et al* (1983), Tervit *et al* (1985), Kiesling *et al* (1986) and Rajkhowa *et al* (1989) who reported it as 7.9,6.0,2.0 and 9.0 respectively. The present finding regarding % egg fertilization rate is lower than the findings of Ahmed and Maurya(1981), Tervit *et al* (1983) and Patil *et al* (1984), who reported it as 81.37,87 and 92% respectively,. The reasons for lower egg recovery and egg fertilization rate is due to the higher incidence of intact follicles and bilateral salpingitis in both the donors from Group IV.

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In-vitro Maturation Of Caprine Oocytes

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ABSTRACT

Oocytes harvested from caprine ovaries post-slaughter were cultured and studied. Recovery rate per ovary was 12.4± 4.6 with 40% good, 40% fair and 20% poor oocytes. 13.75% oocytes after 24hr. culture were in G.V. Stage, 31.24% in maturation stages, 37.50% matured to Met. II and 17.50% showed morphological abnormalities.

* * *

Non-availability of large number of embryos remains a major constraint in large scale embryo transfer programme. Early stages of embryos are also required for micro-mani pulation studies for production of clones/transgenic animals. Superovulation techniques have limitation in this regard. The ovaries of slaughtered animals can be an easy source of oocytes/embryos. Limited work has been done (Zhiming *et al* (1990; Chauhan and Anand, 1991). In the present study, recovery rate, morphology and quality of caprine oocytes were studied.

Materials and Methods

Harvesting and Evaluation of Oocytes : Caprine ovaries collected from a local abattoir in a culture tube containing Dulbecco's PBS were brought to the laboratory within one hour of slaughter. The ovaries were cleaned of the surrounding tissue/bursa with the help of scissors and kept in a petri dish containing Dulbecco's PBS (Himedia) enriched with goat serum. Oocytes were harvested by puncturing the visible ovarian follicles (1-3 mm diameter) with a 18 G hypodermic needle. The medium in the dish was replaced 2-3 times to remove debris before evaluating the oocytes which were examined and graded as good (morphologically normal with complete layers of cumulus cells), fair (morphologically normal

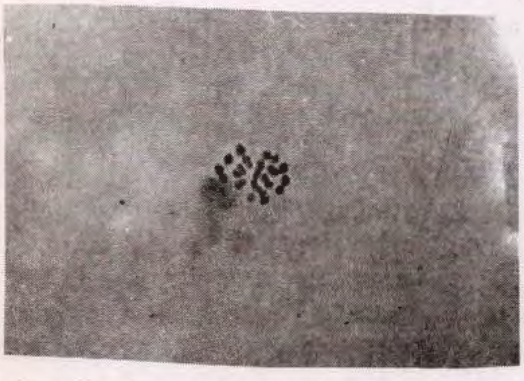

Fig.1. Caprine oocyte at metaphase I stage of meiosis, 640X.

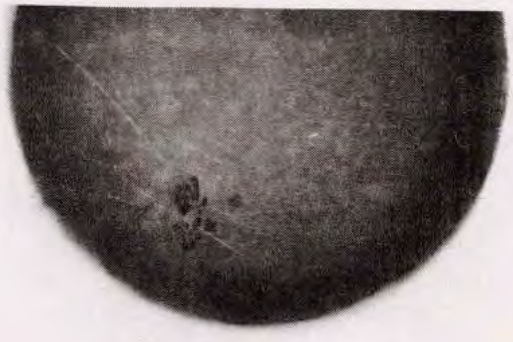

Fig.2. Caprine oocyte at anaphase I stage of meiosis, 640 X.

with incomplete or no cumulus cell layers) and poor (morphologically abnormal with or without cumulus cell layers).

Maturation of Oocytes : Oocytes graded as good were rinsed 3-4 times in harvesting/maturation medium before culture. 10 to 15 oocytes a petri dish containing 5 ml of medium (M 199, Himedia, supplemented with 20% heat inactivated goat serum) were incubated for 24 hours in an atmosphere at 38°C temperature, 95% RH and 5% CO_2 in air. To evaluate the maturation status of oocytes, wet mounting technique (Chang, 1952) and lacmoid staining (Cheng, 1985) were used. Nuclear maturation to metaphase II stage of meiosis was used as criterian of oocyte maturation.

Results and Discussion

The total number of oocytes recovered per ovary ranged from 4 to 24 (Av. 12.4± 4.6). Quality-wise good, fair and poor oocytes were 40%, 40% and 20% respectively. These values were higher to those reported in cattle (Iwasaki *et al*, 1987) and sheep (Agrawal and Polge, 1988). Pigs being a prolific species, a very high recovery rate has been reported (Agrawal and Polge, 1989).

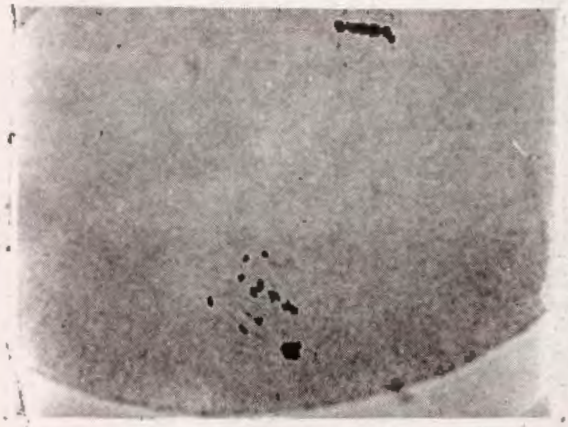

Fig.3. Caprine oocyte at metaphase II stage of meiosis, Polar body 1 is distinct at periphery, 640X.

Out of 160 oocytes examined after 24 hr culture (Table 1), 22 (13.75%) remained in G.V. stage, 50(31.24%) in different stages of maturation (Met I, Ana I, Telo I), 60(37.50%) matured to Met II and 28(17.50%) developed morphological abnormalities (Fig:1-4).

Motlik (1972) obtained 80% maturation rate of pig oocytes using a higher concentration of growth protein (30 mg/ml) in the culture medium. Cheng (1985) reported an average maturation rate of 65.20% when the primary porcine oocytes were cultured at 39°C for 44 to 48 hr in medium 199, supplemented with fetal calf serum and hormone. However, he obtained a higher maturation rate with ovine oocytes when incubated for 24-26hr under similar culture conditions. Maturation rate of sheep oocytes was reduced to 55.34% under similar culture conditions except that the medium used was deprived of external hormones (Agrawal and Polge, 1988). Chauhan and Anand (1991) obtained high - 69% maturation rate of caprine oocytes in Ham's F-12 medium with bovine serum albumin and fetal calf serum. In a report from China, goat oocytes matured to 43% in TALP enriched with new born calf serum, BSA and gonadoropins (Zhiming *et al*, 1990). In the present experiment hormone was not added to

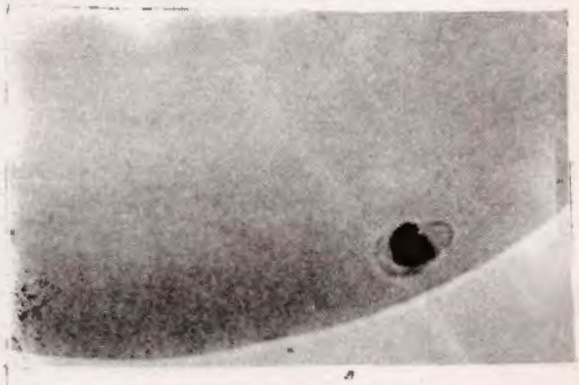

Fig.4. Caprine oocyte with condensed chromatin at periphery, 640X.

Table 1 : **In-Vitro maturation of caprine oocytes.**

S.No.	Parameters	No.	%
1.	No. of trials	12	-
2.	No. of Oocytes examined	160	-
3.	Stages of meiosis		
	a. Germinal vesicle (GV)	22	13.75
	b. Metaphase I (Met I)	33	20.62
	c. Anaphase I (Ana I)	10	6.25
	d. Telophase I (Telo I)	7	4.37
	e. Metaphase II (Met II)	60	37.50
	f. Abnormal	28	17.50

the culture medium. The source of oocytes was also from ovaries of unknown origin collected from abattoir. There is possibility of improving the oocyte maturation rate in subsequent experiments if the interval of animal slaughter and oocyte harvesting time is reduced, oocyte evaluation is done more rigorously before culture and culture conditions are improved.

Acknowledgement

The author thanks the Director of the Institute for providing necessary facilities. The support of USAID is also gratefully acknowledged.

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CASE REPORTS

IJAR:13:2:198-199 :1992

Holocardius Amorphus In Corpus Uteri Of A Buffalo

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Teratogens cause chromosomal mutation and congenital anomalies during neonatal period leading to monstrosities. Metabolic and endocrine imbalances during pregnancy predispose to congenital malformations in foetus. Holocardius amorphus occurred occasionally in cattle (Roberts, 1986). Development of conceptus and placentation usually occurs in single cornua of uterus in bovine twin pregnancy in cattle. Present paper records rare occurrence of bovine pregnancy in corpus uteri and congenital malformation of foetus "Holocardius amorphus" in a buffalo.

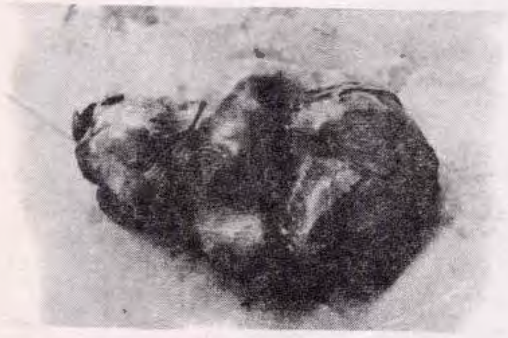

Fig.1. **Holocardius Amorphus delivered by caesarean section**

Fig.2. **Incision in corpus uteri indicating site of prenatal development.**

Fig.3. **Distinctly larger corpus uteri than the uterine cornua evidencing pregnancy in uterine body.**

Fig.4. **Distinctly smaller cornual than corpus uteri caruncles suggestive of migration of foetus.**

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Case Report

A 6 year old Murrah buffalo in its third pregnancy was brought to the University Veterinary clinics with the history of rupture of allantoic sac 72 hrs earlier and cessation of foetal expulsion despite strong uterine and abdominal contractions with lack of udder development. Vaginal examination revealed cervical dilatation and a hard mass without foetal extremities obstructing birth canal suggestive of foetal monster. Obstetrical manipulations proved unsuccessful and foetotomy unsuitable to relieve dystocia. Therefore, caesarian section was undertaken in left lateral recumbency with 30cm. incision paralliel and lateral to the milk vein (Verma *et al*, 1974).

Results and Discussion

Monster was an oval shaped mass without characteristics of foetus, lacked development of hair and was delivered by caesarean section from corpus uteri evidencing prenatal development in corpus uteri (Fig.1,2). Holocardius amorphus confirmed typical structural malformations described by Roberts (1986). Distinctly larger corpus uteri than the uterine cornua in affected buffalo further supported recorded observation of pregnancy in uterine body (Fig.3). Embryonic development and placentation usually occurs in single cornua of uterus in cattle and nongravid horn lacks placental development (Roberts, 1986). Placental development was evident in right cornua of the uterus at necropsy suggesting that implantation and embryonic development occurred in the cornua and conceptus migrated to corpus uteri around mid-gestation in as much as cornual caruncles were distinctly smaller than the corpus uteri of the affected buffalo (Fig.4). Inadequate placental area in corpus uteri could have possibly arrested normal foetal growth predisposing to monstrosity.

Monstrosities often cause dystocia in bovines and obstetrical manipulations and foetotomy were found to be of insignificant value in monstrosities (Parkinson,1974; Bugalia *et al*,1982). Caesarean section was undertaken in the reported Holocardius amorphus monster due to failure of obstetrical operations and unsuitability of foetotomy due to occlusion of birth passage by foetal malformation. Caesarean section was preferred over foetotomy in dystocia due to monstrosities in cattle and buffaloes (Messervy *et al*, 1956; Sharma *et al*, 1984).

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Dicephalus Dipygus Monster In Nagpuri Buffalo

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The birth of monster calf always brings surprise to the common people. Anomalous twins form a graded series from slight duplication to virtually separate individuals and may be classified as free or attached symmetrical or asymmetrical (Potter, 1961). Double monstrosities are relatively more common in the cow, sow, bitch and cat and rare in other animals (Roberts, 1971). A case a Dicephalus dipygus monster buffalo calf is recorded.

Case Report

A Nagpuri she-buffalo aged about 8 years and in fourth parity was presented for dystokia treatment. Her previous calvings were normal. The gestation period was complete with signs of impending parturition. The buffalo was straining hard. The cervix was fully dilated with two foetal hind limbs and one forelimb protruding out, obliterating the birth canal. Attempts to correct the posture and manual removal failed. Hence caesarian section was performed and dead calf removed. The monster was fully developed with one normal head and the other head and neck fused at the level of neck region on the left side of the foetus. Each calf had a pair of forelimbs and hind limbs (total eight limbs), tail and anal opening with three ears and three eyes (Fig.1).

Post-operative recovery was uneventful and the buffalo started giving 4 litres milk per day, three days following treatment.

Fig.1. **Dicephalus dipygus monster**

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Dicephalic, Dicardiac Monster In Dangi Cow

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Developmental abnormalities of the ovum, embryo or foetus occur in all the species of domestic animals. The severe ones can cause resorption, abortion, mummification or stillbirth. Less severe forms result in structural abnormalities leading to monstrosities, stillbirth or dystocia (Sane *et al*, 1982).

Duplication of cranial as well as of caudal foetal parts has been reported by Roberts (1971), the former being more common. The present report is about duplication of cranial portion of foetus in Dangi cow.

Case History

Six years old Dangi cow with dystokia was presented at College Veterinary Polyclinic, on 19-1-91. She had given a normal calf previously. P.V. examination revealed that calf was in posterior longitudinal presentation; dorso-sacral position with adduction of right stifle joint. Manual correction of right stifle was carried out and calf was pulled out gently upto its neck. Due to resistance at the neck region, gynaec examination revealed two distinct heads with necks. The calf was removed with suitable mutation. It was alive for a very short time.

Foetus : The monster was a well developed female foetus (Fig. 1), with two heads joined to the thorax by two separate necks which were well developed. One head had a fissured right nostril.

Autopsy revealed complete bifurcation of vertebral column till the first thoracic vertebra. Thereafter second and third thoracic vertebrae were large sized due to union of vertebral column, followed by a single vertebral column.

Fig.1. **Dicephalic monster of Dangi cow.**

Fig.2. **Duplicate heart.**

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2. Physician, Veterinary Polyclinic.
3. Assistant Professor of Anatomy.

The musculature and vertebrae of each neck were well developed. The brains were normal, both the spinal cords left the cranium separately and were united at the level of third thoracvertebra.

Heart was double. One heart was of a normal size and other about 2/3rd of the former (Fig. 2). Both the hearts were united at the atria. Right auricle of larger heart was united and communicated with the auricle of the other. The normal size heart had all the four chambers, while the smaller one had only right auricle and ventricle internally. Externally it showed demarcation between right and left side but internal longitudinal septum was absent. The right auricle of the normal heart communicated with that of smaller heart. The right and left auricles of the normal heart had a patent foramen ovale (Fig. 3). Anterior vena cava bifurcated and entered in the respective right auricles. Pulmonary artery of the small heart was closed, but that of

Fig.3. **Patent foramen ovale (F).**

the normal heart communicated with aorta through the patent ductus arteriosus.

The lungs did not show duplication. The trachea was duplicate and entered in respective lungs separately without bifurcation. The two oesaphagii opened into rumen by two openings at rumino-reticular groove. Other abdominal organs did not show duplication.

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Male Pseudo-hermaphroditism In A Cow-calf - A Report

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A case of male pseudo-hermaphroditism in a cow calf having a typical malformation of genitalia is reported.

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An intersex, genetically a mixture of male and female, is an animal with congenital malformations of sexual development that confuse the diagnosis of sex (Hafez, 1987) and

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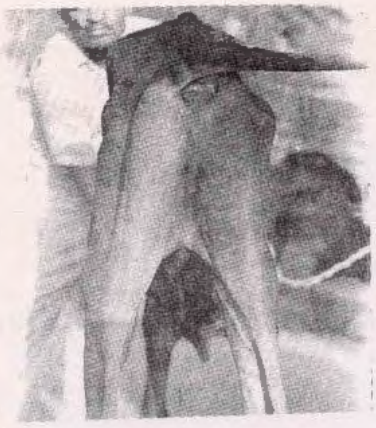

Fig.1. **Note absence of Scrotum**

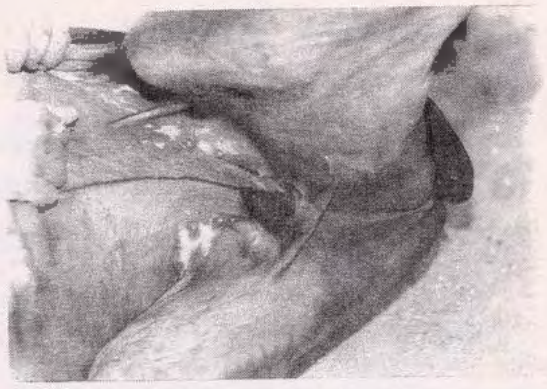

Fig.2. **Note rudimentary penis**

that causes infertility and sterility (Sane *et al* 1982).

Case Report : A non-descript cow-calf of 1 1/2 years age, was brought to the Veterinary Polyclinic for cheek-up of abnormal appearance of the genitalia.

Clinical Observations : The general health and growth rate of the animal was normal. Moreover it was alert and active. All the physiological functions and anatomical features were also found to be normal except the congenital malformation of the genitalia. Close examination revealed absence of scrotum and penis (Fig. 1). The prepucial opening was present at the level of normal location of scrotum. Though the scrotum was absent, two testicles could be palpated in the scrotal region beneath the skin, just in front of the abnormal prepucial opening. A short rudimentary penis was palpable near ischeal arch of about one inch length from its base (Fig. 2). Obviously, the sigmoid flexure and full length penis was absent. However, the animal could urinate normally without any difficulty. The dam and sire were normally bred animals, without any history of defective progenies in the past.

The case can be described as a male pseudo-hermaphrodite cow-calf, which is in consonance with the observations of Sane *et al* (1982).

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Congenital Uro-genital Defect In A Calf

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Congenital anomalies of the urinary system are not uncommon (Roberts, 1977). This paper reports a congenital anomaly of absence of anal opening, penis, preputial sheath with imperfect closure of urethra in a nondescript cow calf and its successful treatment.

A 6 day male cow calf was presented to the Veterinary College Polyclinic. With the complaint of absence of anal opening and dribbling of urine through the opening ventral to anus. Detailed examination of the calf revealed absence of anus, penis and preputial sheath. Both the testicles were retained in the inguinal canal and were palpable near the external inguinal ring. There was remnant of external urethra in the form of groove covered with pinkish coloured skin (Fig. 1). The calf was passing small quantity of urine through the undeveloped external urethra, ventral to the anus.

The anal opening was made surgically under local analgesia as described by Oehme and Prier (1974). The urethral opening was made larger and an appropriate size indwelling PVC catheter was placed to avoid the structure and further narrowing of urethra.

Absence of penis, preputial sheath and anus in this calf may be due to failure of elongation of genital tubercle (Aery,1962). Vershney and Prakash, (1990) reported congenital abnormal bifurcation of two separate scrotum with urethral opening ventral to the anus. Atresia ani is recorded as a congenital defect in calves (Cho and Taylor, 1986). Therefore absence of anal opening, penis and preputial sheath existed in the case under report could be a congenital anomaly.

Fig.1. Showing remnant of external urethra in the form of groove covered with skin .Note the absence of penis, preputial sheath & opening of urethra ventral to anus.

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Perosomus Elumbis Monster In A Doe - A Case Report

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Perosomus elumbis occurs in ruminants and swine. The primary abnormality in this type of monster is hypoplasia or aplasia of the spinal cord of fetus which ends in the thoracic region. The regions of the body including the hind limbs which are supplied by the lumbar and sacral nerves, exhibit muscular atrophy and joint movement does not develop (Arthur *et al*, 1989).

A non-descript Doe aged about 2 1/2 years attended the Veterinary College Clinic, Namakkal with a history of straining since previous night. Vaginal examination revealed fully dilated cervix and fetus presented the head and ankylosed hind limbs. After proper lubrication and traction, a dead kid (Wt. 1.75 Kg) was delivered.

The spinal cord was developed only upto the throacic region with a depression caudal to the throacic vertebrae and a flat bone in place of the lumbar and sacral bones. The monster had a small flattened deformed pelvis and there was ankylosis of the fore and hind limbs (Fig 1). Muscular atrophy of the rear quarters was also observed. The monster was a typical perosomus elumbis as per the classification of Roberts (1971) and Arthur *et al* (1989).

Acknowledgement

The authors thank the Dean, Veterinary College and Research Institute, Namakkal for successful conduct of this study.

Fig.1. Perosomus elumbis monster.

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Monocephalus Dipygus Conjoined Twin Lamb

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A case of monocephalus dipygus conjoined twin lamb in a sheep was recorded.

A primiparous non-descript sheep, due for lambing, was presented to the Veterinary Dispensary with a history of dystokia. Water bags were ruptured and foetal limbs were present in the vaginal passage of the sheep. There was constant straining since past 3 hours.

P.V. examination revealed four limbs in the birth canal, of which three were fore limbs and one hind limb of foetus. It was diagnosed as a case of monstrosity. Caeserean operation was performed by Young's modified approach under sedation and local infiltration anaesthesia.

A live female monster with one head and two abdomens was removed. It died immediately after birth. Duplication of parts were seen from thoraco-sternum onwards, with 8 limbs and a distinct clear duplication of abdominal and pelvic parts.

On scanning the available literature it was revealed that similar case is not yet recorded in sheep. Roberts (1971) reported that the conjoined twins arise from a single ovum and are monozygotic.

It is also reported that duplication of cranial part of the foetus is more common than that of the caudal portion, but in present case there was duplication of caudal portion of the lamb. It was confirmed as a case of Monocephalus Dipygus Conjoined twin lamb.

Monocephalus Dipygus Conjoined Twin Lamb

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WILD LIFE

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Observations On Reproductive Behaviour Of Tigress (Panthera tigris) In Captivity

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Reproduction in wild life in captivity is a very complicated affair. Sexually mature tigress and tigers in captivity were studied for certain aspects of reproduction with twenty three births in eight litters.

Materials and Methods

Sexually mature four tigress and five tigers of Maharajbag Zoo, Nagpur were observed. Data extending over a period of twenty-one years was studied pertaining to certain aspects of reproduction such as age at sexual maturity, oestrus, oestrus behaviour, sexual behaviour, courtship/copulation, gestation period, parturition, litter size, birth weights and life span. All the animals were maintained under uniform managemental practices with one tigress and tiger together per cage. Sand bed kept wet by sprinkling water twice a day during hot season for maintaining proper temperature to avoid heat stroke was also provided in each cage.

Observations and Discussion

Sexual maturity : The sexual maturity attained by two tigress in captivity was at the age of 3.3 and 3.4 year respectively, which is in agreement with Kleiman (1974) and Prater (1990), who opined that early sexual maturity was attained in tigress in captivity.

Oestrus and Oestrus behaviour : The average oestrus period varied from 3 to 7 days. (Av. 4.7 days). Kleiman (1974) reported mean duration of oestrus in tigress as 7.2 days.

Tigress is polyoestrus animal and breeds throughout the year. Out of four tigresses, two (50%) exhibited oestrus twice a year, whereas one (25%) exhibited thrice a year and one (25%) only once a year. Most tigresses exhibited heat from July to September, followed by October to February and least from March to June. It was observed that if litter was lost, the frequency of oestrus increased to three times a year, which is in full agreement with the observations of Majupuria (1990).

Sexual behaviour : Tigress in oestrus was off feed and uneasy for 2-3 days and sought the company of tiger. Vulval lips were moist, turgid and swollen with frequent micturition. Agressiveness to zoo staff was prominantly noticed during this period.

Courtship and coitus : Tigress in heat manifested intense signs of courtship rubbing cheeks, licking the ears, face, body and flank

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region of the male, followed by typical mating call during the courtship. The frequency ranged between 40 to 70 times a day with copulation interval of 5 to 15 minutes. The average copulation ranged 4 to 5 times per hour. The frequency was minimal(1 - 1 1/2 hour) on first and last day of oestrus. The duration of copulation was around 2 minutes, which is in partial agreement with the observations of Lee Server (1991).

Gestation period : The average gestation period recorded in two tigresses for three conceptions was 105.6 (range 104 to 107) days. The gestation period is 100-108 days (Crandall, 1965) and 113 days (Asdell, 1964). The difference in gestation period may be due to variations in methodology followed.

Parturition : The interval between parturitions in tigress of 4th parity was : 4 mths: 5m. 22 d.; 5 m 15 d and 19 mths (range 4 to 19 months). This is in partial agreement with Acharjyo (1970) who stated that the interval between parturitions could be minimised if the tigress was allowed to remain with her mate post-partum.

Total births : 23 births in 8 litters in 4 tigresses were recorded, of which there were three still-born cubs in one litter. Acharjyo (1969) reported similar observations. Amongst 23 births, 13 (56.5%) female and 10 (43.5%) male cubs were born. The sex ratio at birth in tigress is nearly equal (Majupuria, 1990).

Litter size : The number of cubs born per litter ranged from 2 to 4 (Av. 2.87) per litter. The cubs were born with closed eyes at birth which opened between 9 to 11 days. This is in accordance with Asdell (1964) and Crandall (1965).

Birth weights : The birth weights of female and male cubs taken immediately after birth varied from 1 to 1 1/2 kg for female and 1 1/2 kg for male cub, which is in agreement with Acharjyo (1970).

Life span : One tigress in captivity born on 25.11.61, died on 22.11 82 with a life span of 21 years in captivity. This finding is in agreement with Prater (1980).

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