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Editorial

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INDIAN JOURNAL OF ANIMAL REPRODUCTION (ISSAR) ANNOUNCEMENT

The newly elected office-bearers of ISSAR with Dr. A. S. Kaikini as President will be functioning from 1st January 1993 onwards. Consequently, the following changes will be effective from 1-1-93.

1. **IJAR 13 (2) : DECEMBER 1992** will be the last IJAR issue to be published from Nagpur edited by Dr. A. S. Kaikini.

2. IJAR Annual Subscription will be Rs. 200/-.

3. The present rates of advertisement will apply to one insertion only, instead of per Volume (two insertions).

4. **IJAR 14 (1) : JUNE 1993** and onwards will be published from Madras under the editorship of Dr. S. R. Pattabhiraman, Head, Department of Clinics, Madras Veterinary College, Vepery, Madras-600 007, to whom all IJAR correspondance be addressed w.e.f. 1-1-93.

All ISSAR Members IJAR contributors, subscribers and advertisers are requested to note the above change.

June 30, 1992.

D.R.Pargaonkar	A. S. Kaikini	A. R. Rao
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IJAR:13:1:1-4:1992

Embryo Transfer For An Effective Break-through In Livestock Production*

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INTRODUCTION

Livestock production plays a very vital role in our National economy. Animal Husbandry is gaining importance due to its crucial rural agrarian economy by providing the much needed animal protein food (meat, milk, eggs), fibre, motive power, dung (organic manure) with relatively greater gainful employment to small and marginal farmers, agricultural labourers and the rural poor population below the poverty line (BPL). The contribution of livestock to Gross National Production (GNP) is phenomenal–around Rs.2,00,000 m. per annum, 80% of which is from cattle and buffaloes. Livestock have a much larger contribution to manpower employment constituting a vital element in rural development programme (RDP).

As per 1982 Census, India possesses 184.86 m. cattle, of which nearly 10 m. are cross-breds and 61.05 m. buffaloes. The contribution of buffalo milk is very significant in comparison to Asia (68.06%) as well as the World (65.05%). Besides, there are 96m. goats, 48m. sheep, 2m. equine, 1m. camel, 10m. pigs and 193m. poultry enriching national economy.

The genetic diversity of livestock is reflected in the number of Descript breeds thus: Cattle 26, buffaloes 7, sheep 40 and goats 20, besides a no. of camel, equine, pig and poultry breeds. Our cattle breeds of dairy, draft and dual purpose types are well adapted to tropical heat, poor feed resources and disease resistance. India has World's best dairy buffalo breeds and has provided superior dairy buffalo germ plasm all the World over. Besides, we have carpet wool breeds of sheep and prolific breeds of goats. Thus there is an urgent and imperative need to maintain genetic diversity and take suitable steps to conserve the breeds likely to become extinct. Herein, embryo transfer technique (ETT) together with Frozen semen technology is rightly indicated.

By and large, the rural cattle population is of Non-Descript (ND) type, with an assorted genetic make-up due to indiscriminate breeding in the past. ND cattle mature late, are erratic breeders, poor/scanty milk yielders with an equally narrow lactation period and a very wide dry period. This scenerio is rapidly changing dne to the implementation of Cross-breeding programme of low producing and ND cattle with high yielding exotic dairy breeds on a massive scale all over the country since the past 25 years, initially by A.I. using chilled semen and now with frozen semen of exotic (Jersey, Holstein-Friesian, Brown-Swiss, Red Dane) bulls.

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

* Chairman's address at the Technical Session on "Livestock Production-Embryo Transfer" at the DAE Symposium organised by Food & Agri. Committee, Board of Research in Nuclear Sciences, GOI Department of Atomic Energy & BAIF Research Institute of Animal Health, at the University of Poona, Pune 411 007, February 22-24, 1992.

The outstanding work done in this regard giving a total impact on rural development through cross-breeding and boosting livestock economy, by the Bharatiya Agro Industries Foundation, (BAIF), Uruli-Kanchan over the past 25 years under the dynamic and innovative leadership of Padmashri Dr. Manibhai Desai and his team of dedicated Scientists, is without any parallel. It is indeed a wonderful coincidence that the present DAE Symposium Synchronizes with BAIF Silver Jubilee.

Impact of Frozen Semen Technology

The real boost for livestock production came from Frozen Semen Technology which is by far the best gift of Scientific research to mankind. Its greatest potential lies in the fact that it has achieved the hitherto impossible task of disseminating the best available bull germ plasm in its potent form any where in the world, breaking the 'distance' barrier between the donor bull and recipient cows around the globe. Livestock productivity is thus benefitted by the ever-increasing "Biotechnological" explosions, latest being Embryo Biotechniques.

Biotechnology in Livestock Production

The term Biotechnology is currently being used to denote a wide range of biological manipulations such as cell and tissue culture, embryo transplantation, DNA material transfer across sexual barriers, microbiological enrichment of cellulose material, fermentation and various forms of bio-mass utilisation. The hardcore of biotechnology is re-combinant DNA technology resulting in transgenic micro-organisms, plants and animals. The results of gene engineering research in Veterinary & Human medicine are evident in the production of growth producing (Somatotropin) hormone, insulin, interferone and different kinds of vaccines. Likewise is the case of production of renin used for cheese manufacture.

Embryo Transfer

Embryo transfer is a method of artificial breeding, whereby newly formed embryos are removed prior to implantation from a female donor animal and transferred into the uterine tract of another female (recipient) of the same species where they develop to term. The resultant off-springs derive their genes from the donors and males to which the donors were bred.

Embryo transfer technique (ETT) for faster multiplication of genetically superior highly productive animals (cattle, sheep, pigs) is extensively practiced on a commercial scale in USA, Canada and Australia. ETT helps in maximum utilisation of the female germ plasm (Ova) of genetically superior donor cows, achieve rapid multiplication of breeds,increase the reproductive capacity through twinning, transfer of embryos of superior breeds to inferior (ND) ones and ease of export and import of embryos between countries, obviating the hazards of physical transhipment of live animals. This technique briefly consists of

1. Selection of genetically superior high milk yielding donor cow and genetically inferior low milk yielding recipient cow. Heat (oestrus) of both these cows is synchronized with prostaglandins.

2. Superovulation is effected in the donor cow by injecting FSH (Follicle Stimulating Hormone)

3. Donor cow is inseminated with frozen semen of genetically superior top pedigreed or Progeny tested bull.

4. Fertilised Ova (embryos) are collected from the uterus of donor cow, seven days after A.I. by flushing with a two way Foley Cathetor using special nutrient medium.

5. Isolating good embryos from uterine flushings after detailed morphological ex-

amination under stereo-microscope. Normally, six embryos per flush are available.

6. Maintaining isolated embryos in the special nutrient medium at 37°C in an incubator.

7. Transfer one embryo in the uterus of recipient cow which serves as a Surrogate / foster mother for the genetically superior embryo developing in her uterus (incubator mother).

Freezing of Embryos can be done successfully and stored in Liquid Nitrogen (-196°C) for posterity.

Limitations of ETT in Livestock Production

Under Indian conditions, limitations of ET are :

1. Paucity of progeny tested bull frozen semen.

2. Genetic boost from maternal side is less vis-a-vis the sire.

3. Expensive due to low survival rate of Embryos.

Useful research data on ETT is available from National Dairy Research Institute, Karnal; Indian Veterinary Research Institute, Izatnagar and National Institute of Immunology, New Delhi. Planning Commission has named National Dairy Development Board (NDDB) Anand as the nodal agency for field implementation at the doors of farmers.

Research & Development (R & D) must go hand in hand. Certain State Agricultural Universities have done nucleus work in this direction e.g. PKV, Akola and KKV, Bombay. Adequate financial and resource support from Central Govt., ICAR, DST is essential.

Punjabrao Krishi Vidyapeeth, Akola is the first amongst the Agricultural Universities in Maharashtra to undertake the Pilot project on "Embryo Transfer Technology" with trials conducted in cross-bred cows in the year 1988-89 and 89-90. For this effort, collaboration with National Institute of Immunology, New Delhi was established. Presently three calves born of ETT are produced and maintained at LIF., Akola. Incidently, a twin female calves born out of ETT may be the first of its kind in the country.

Synchronisation of oestrous in Buffaloes:

Buffaloes being seasonal breeders their calvings take place during certain period of the year. In off season many of the buffaloes have silent heats, which go undetected. This results in longer dry period and longer inter-calving period in buffaloes. The dry non-pregnant buffaloes become financial burden on the farmer. Hence, such buffaloes are sold for nominal price or sent to the slaughter house. The net result of this, is the rapid decrease of valuable buffalo germ plasm. Due to the seasonal breeding pattern of buffaloes, buffalo milk is amply available in winter, while there is acute shortage in summer.

There are many buffalo owners in this region who purchase pregnant or lactating buffaloes at higher price from Haryana and sell them at throw away price when the animals go dry. Therefore, the margin of profit is very less to these cattle owners.

As a solution for this problem, synchroni-sation of estrus by using hormones was tried in buffaloes to induce oestrus in them at desired time. This experiment was conducted on Livestock Instructional Farm, Akola and in the Village Vanoja and Ugava of Akola District. In this experiment 100 per cent of the treated buffaloes were synchronised and 48 percent of the buffaloes conceived or at least brought to cyclicity to get covered in due course.

Private organisations such as Raymond's E.R.C. at Gopalnagar, Bilaspur (M.P.) have achieved considerable commercial success in livestock production with ETT. Coordination at the National level is essential, for which ICAR should take the lead.

ETT is the modern technique of tomorrow for which we must be prepared. Like A.I., ET shall have to be implemented in the field by Veterinarians working in State Animal Husbandry programmes. Training should be imparted to these personnel as a phased programme.

Special courses on ETT and related Biotechniques in reproduction should be formulated and implemented in the BVSc and AH Degree programmes in all Veterinary colleges.

Planning Commission has recently decided that under the 8th Five year plan, each State will have a separate University of Veterinary Science and Animal Husbandry, which is a positive step in the right direction. Such vital changes can be implemented by these Universities, emulting the excellent example set by the Tamilnadu University of Veterinary and Animal Sciences, set up in 1988 at Madras.

Embryo transfer is essential for enhancing livestock production and particularly for conservation of indigenous breeds and endangered species, besides developing 'Elite' herds. Intensive efforts in this direction are therefore indicated.

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Prostaglandins In Female Animal Reproduction: A Review

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Prostaglandins (PGs), mainly PGE_2 and PGF_2 alpha have been implicated in the physiological regulation of various events in the reproductive organs of domestic animals. Their involvement in various reproductive processes and the modification of these processes by exogenous PGs is a major area of research. In the field of female reproduction there has been a 'publication explosion' on the role of prostaglandins, particularly PGF_2 alpha. Several studies indicate that PGs are involved in the mechanism of oocyte maturation, ovarian steroidogenesis, ovulation, luteinization, luteolysis, secretion of gonadotrophins from the adenohypophysis, tubal contractility, transport of zygote, implantation, uterine contractility, cervical ripening and parturition (Karim & Hillier, 1979; Bygdeman, 1981).

However, from the application point of view perhaps the most important discovery is the role of PGF_2 alpha in causing luteolysis in many species of domestic animals. PGF_2 alpha is considered to be the primary uterine luteolysin in sheep (Goding, 1974) and good evidence exists for a similar role in cow (Nancarrow *et al*, 1973; Kindahl *et al*, 1976), pig (Glesson *et al.*, 1974) and probably in several other mammals (Horton & Poyser, 1976; Knickerbocker *et al.*,1988). In the cycling cow pulsatile secretion patterns coincide with luteolysis (Kindahl *et al.*, 1976; Betteridge *et al.*, 1984) and PGF_2 alpha as well as its synthetic analogues induce luteolysis when injected between days 5 and 16 of the cycle

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(Hansel & Schechter, 1972; Rowson *et al.*, 1972; Schams & Karg, 1982). Furthermore, active or passive immunization against PGF_2 alpha prolonged the luteal function in normally cycling sheep (Scaramuzzi and Baird, 1976; Fairclough *et al.*, 1981) and cattle (Fairchough *et al.*, 1981; Chang *et al.*, 1987) and extended the lifespan and function of corpora lutea anticipated to be short-lived in pre-partum and postpartum cows (Copelin *et al.*, 1989). The luteolytic property of PGF_2 alpha and its synthetic analogues has several applications in the field of female reproduction as reviewed below.

(i) Control of the oestrous cycle:

The use of exogenous PGF_2 alpha for the synchronization of oestrus in cattle was first reported in 1972 (Liehr *et al.*, 1972; Louis *et al.*, 1972; Rowson **et al.**, 1972) and has been subsequently confirmed by several other investigators (Stabenefeldt *et al.*, 1978; Hansel and Beal, 1979; Downey, 1980). Initial studies reported that PGF_2 alpha given in two daily doses (0.5 - 1.0 mg) into the uterine horn ipsilateral to the ovary containing the corpus luteum (CL), induces luteolysis with oestrus occuring after 48 to 85 hr (Hansel and Schechter, 1972; Rowson *et al.*, 1972; Shelton, 1973; Hearnshaw *et al.*, 1974; Smith, 1974). However, intra-uterine administration requires patience and skill. The intravaginal (Louis *et al.*, 1973) and intravulvo-submucous routes of administration (Ono *et al.*, 1982) have also been tried.

Subsequent reports, deal with response to a single injection (subcut or I.M.) using higher dose levels of 20 to 30 mg of PGF_2 alpha Tham preparation or one of its potent analogues (Lauderdale, 1972, 1975; Cooper, 1974; Schams & Karg, 1982). However, exogenous PGF_2 alpha is not effective in altering the bovine oestrous cycle if administered before day 5 or beyond day 16 of the cycle (Cooper, 1974) and for that reason nearly 30% of cycling cattle may fail to be synchronized by a single injection. Two regimens have been developed to ovecome this problem. In one regimen, all animals are treated with two doses of PGF_2 alpha given 11 days apart (Cooper, 1974) and inseminated at the oestrus following the second injection. In the second regimen cows are bred at natural oestrus during a 5 day period; then the remaining animals are treated with PGF_2 alpha on day 6 and bred at the induced oestrus. Several variations of these treatment regimens have been reviewed (Cooper, 1981; Lauderdale *et al.*, 1981; Wenkoff, 1986). In general, double injection schedule gives more rapid and precise response in the synchronization of oestrus, LH release and ovulation (Cooper, 1974; Leaver *et al.*, 1975). More recent approach is to give a single injection of PGF_2 alpha after a short term (5 to 7 days) progestagen treatment (Hansel and Fortune, 1977; Hansel and Beal, 1979).

Fertility in heifers and suckling beef cows at the synchronized oestrus after a single (Lauderdale *et al.*, 1974) or double injection schedule (King and Robertson, 1974; Hafs *et al.*, 1975; Leaver *et al.*, 1975) appears to be normal presumably due to a normal endocrine profile at PGF_2 alpha induced luteolysis (Cooper and Rowson, 1975; Dobson *et al.*, 1975). However, in lactating dairy cows fertility at the PGF_2 alpha synchronized oestrus is generally lower than in heifers bred at synchronized oestrus (Hafs *et al.*, 1975, Leaver *et al.*, 1976) or control cows bred at natural oestrus (Leaver *et al.*, 1976; Roche, 1976). Summarizing the results of several field trials on the use of various PG regimens in heifers, beef cows and dairy cows, Cooper (1981) states, "A feature of these data is the variability of fertility, not only between farms, but from year to year and even from season to season, on the same farm. It is the respon-

sibility of the practising veterinarian to recognise this variability and to be able to identify those circumstances in which postaglandins can be used most profitably".

In the buffalo, PGF_2 alpha and its analogues have been tried for oestrus synchronization using single (Jainudeen 1976; Pant and Singh, 1980; Rajamahendran *et al.*, 1980) as well as double injection schedule (Kumaratillake *et al.*, 1977; Perera *et al.*, 1977; Prasad *et al.*, 1978, 1979 a, b; Rao & Rao, 1978; Pathiraja *et al.*, 1979). The results are nearly similar to those reported in cattle. Also, the endocrine changes following PGF_2 alpha induced luteolysis appear to be similar to natural oestrus (Kamonpatana *et al.*, 1979; Bachlaus *et al.*, 1980). However, these studies are based on limited number of animals and there is a shortage of field trials.

PGF_2 alpha and its analogues have also been employed for the synchronizaton of oestrus in mare (Allen, 1981; Bristol, 1986), Sheep (Douglas and Ginther, 1973; Fukui and Roberts, 1977; Haresign and Acritopoulou, 1978; Hackett and Robertson, 1980), goat (Ott et al., 1980; Bretzlaff *et al.*, 1981; Thimonier, 1981) and pig (Guthrie, 1975; Guthrie and Polge, 1976). However, in the pig PGF_2 alpha is not luteolytic until day 12 of the cycle (Diehl & day, 1974).

ii. Treatment of unobserved oestrus and sub-oestrus:

In dairy cows, up to 40% of oestrus periods may pass undetected (Williamson *et al.*, 1972; King *et al.*, 1976). Several studies have revealed the effectiveness of PGF_2 alpha for cattle with unobserved oestrus (Cooper *et al.*, 1976; Eddy, 1977; Seguin *et al.*, 1978). Results from 886 cows in six trials revealed that 67% (range 53% to 86%) cows were observed in oestrus within six days of treatment with a luteolytic dose of PGF_2 alpha or one of its analogues. The failure of some cows to respond was principally due to errors in identification of responsive CL and to unobserved oestrus after treatment. Pregnancy rates of treated cows inseminated in the induced oestrus averaged 54% (Seguin, 1981).

In the buffalo, where sub-oestrus is major problem, PGF_2 alpha has been used with reasonable success (Rao & Rao, 1979; Singh *et al.*, 1979; Khurana *et al.*, 1981; Chauhan *et al.*, 1982; Pant & Singh, 1991). In the study of Rao & Rao (1979), 34/36 (94.4%) sub-oestrus buffaloes showed a visible heat within three to four days of treatment with cloprostenol. The fertility at induced oestrus (44%) was lower than that reported earlier in normal cycling buffaloes (52.5%) in which oestrus was synchronized with cloprostenol (Rao & Rao, 1978). In the study of Singh and associates (1979). 88.3% sub-oestrus buffaloes exhibited oestrus following treatment with 25 or 30 mg PGF_2 alpha (Prostin, Upjohn) but only 36.8% conceived at the induced oestrus. Others have reported conception rates of 44% (Singh *et al.*, 1979), and 28.8% (Khurana *et al.*, 1981) following A.I. at the induced oestrus. However, in our study we obtained a conception rate of 54.8% following natural service at the PGF_2 alpha induced oestrus (Pant and Singh, 1991).

iii. Induction of abortion:

The luteolytic property of PGF_2 alpha and its analogues has been effectively employed for removal of unwanted pregnancy in heifers (Schultz and Copeland, 1981) and for termination of pathologic pregnancies like fetal mummification, hydramnious, hydrallantois and prolonged gestation (Vandeplassche *et al.*, 1974, Talbot and Hafs, 1975; Barth, 1986). Pregnant heifers appeared to be more responsive to PGF_2 alpha–Tham salt prior to 120 and after 260 days of gestation. This observation is based on 100 per cent success rate with doses of 45 mg (up to 120 days) or 15 mg or greater

(260 days or greater). However, it is less effective (66%) during 150 to 250 days even with doses of greater than 45 mg (Lauderdale, 1975). During 6th to 8th months of gestation, additional treatment with a glucocorticoid viz., dexamethasone (dose 25 mg) is required to increase efficacy to acceptable levels, while in the final month of gestation either PGF_2 alpha or glucocorticoid treatment alone will induce parturition (Barth, 1986). Among the synthetic analogues 375, ug Cloprosterol injected intramuscularly is an effective and safe abortifacient up to 150 days of gestation in feedlot heifers (Copeland *et al.*, 1978). Although there is lesser demand for induction of abortion in other domestic animals (Schultz & Copeland, 1981), however, PGF_2 alpha and its analogues have also been employed with varying success for the induction of abortion in mare (Kooistra & Ginther, 1972; Douglas *et al.*, 1974, Squires *et al.*, 1980), goat (Holst and Nancarrow, 1975) and pig (Diehl & Day, 1974).

iv. Induction of parturition:

In cattle, parturition can be induced between 36 and 48 hr after intravenous, subcutaneous, intrauterine or intramuscular administration of a luteolytic dose of PGF_2 alpha or its analogue (Johnson, 1981). Greater fetal prematurity was associated with longer intervals (Carter and Parsonson, 1976; Day, 1979) and high rate of placental retention (Day, 1979; Plenderleith, 1978). Elective parturition with PGF_2 alpha can also be induced in mare (Rossdale *et al.*, 1979), sheep (Harman & Slyter, 1974), goat (Umo & Fitzpatrick, 1976) and swine (Einarsson, 1981). In swine when parturition was induced before day 111 of gestation, high neonatal losses resulted (First and Bosc, 1979). However, injection of PGF_2 alpha (10 mg) on day 112, alongwith relaxin (600 U) on day110 or days 110 and 111 of pregenancy, remarkably improved the synchrony of farrowing (Butler and Boyd, 1983). Induced farrowing may reduce the incidence of MMA syndrome (Einarsson *et al.*, 1975).

v. Hastening uterine involution and prevention of retention of placenta:

In the cow, increased peripheral concentrations of 15 keto -13, 14-dihydro-prostaglandin F_2 alpha (PGFM)- a metabolite of PGF_2 alpha - observed at normal calving also extend into the post-partum period (Edqvist *et al.*, 1978, Lindell *et al.*, 1982). This rise in PGFM is positively correlated with the rate of uterine involution in cows with normal parturition (Edqvist *et al.*, 1978; Eley *et al.*, 1981). Accordingly, exogenous administration of PGF_2 alpha has been shown to promote uterine involution in the cow presumably due to its nterotonic effect (Lindell & Kindahl, 1983). However, this study was conducted in only three cows employing a very high dose schedule (25 mg per day twice daily from day 3-13 after parturition). In an earlier study, high repeated doses of PGF_2 alpha caused a refractoriness of the uterine musculature (Eiler *et al.*, 1981) Also, in other study partial blocking of the endogenous secretion of PGF_2 alpha, with or without supplementation of PGF_2 alpha, did not affect the rate of uterine involution (Guilbault *et al.*, 1987). Clearly more studies are needed before PGF_2 alpha can be recommended for enhancing uterine involution.

The uterotonic effect of PGF_2 alpha in the non-pregnant cow (Eiler *et al.*, 1981) has also been employed for the prevention of retained fetal membranes consequent to induced calving (Gross *et al.*, 1986). The treatment (10 mg PGF_2 alpha) was only effective if given at one hour post-calving. On the other hand, fenprostalene an analogue of PGF_2 alpha with a longer half life of 18 to 23 hr was effective over the range of treatment interval which was 11 to 56 hr post-partum (Herschler and Lawrence, 1984). This is in agreement with the sugges-

tion that a deficiency of PGF_2 alpha or a lack of conversion of PGE to PGF_2 alpha may account for retention of placenta (Leidl *et al.*, 1980; Horta, 1981; Gross *et al.*, 1985). According to Gross (1987) placental production of E series PGs predominated in cows with retained placenta while in cows that expelled placenta normally, F series PGs predominated in placenta. However, others have observed high PGFM levels in cows suffering from retained placenta (Bosu et al., 1984; Matton *et al.*, 1987) presumably due to conversion of PGE synthesized by Fetal membranes (Williams and Gross, 1981) to PGF_2 alpha in the uterus (Guilbault *et al.*, 1984). The uterotonic effects of prostaglandin are minimal except when associated with luteolysis (Guay & Lamothe, 1980) and accordingly there appears to be no physiological basis for prostaglandin treatment in the immediate post-partum period (Bosu et al., 1984). Nevertheless, PGF_2 alpha may be useful in the treatment and prevention of retention of placenta under some situations. Further work is required to ascertain if PGF_2 alpha can be routinely recommended for this purpose in uninduced calvings (Wichtel, 1991).

vi. Treament of pyometra and endometritis :

PGF_2 alpha has been used for the treatment of either post-partum or post-service bovine pyometra. Majority of cows (about 90%) exhibit oestrus and begin to discharge pus within 3 to 5 days after the luteolytic dose of PGF_2 alpha (Gustafsson et al., 1976) or its analogue (Jackson, 1977) with complete uterine evacuation by day 12 to 14 (Ott and Gustafsson, 1981). Interestingly, even a sub-luteolytic dose (5 mg) of PGF_2 alpha was 60 to 80% effective (Gustafsson et al., 1976). Fazeli and co-workers (1980) reported that 95% dairy cows with post-partum pyometra evacuated the uterus after one or two treatments with a PGF_2 alpha analogue (Cloprostenol) given 7 to 10 days apart and supplemental antibiotic therapy was not beneficial. Several other studies have demonstrated PGF_2 alpha to be the most effective therapy for pyometra in the bovine (Cooper et al., 1976; Schultz, 1978; Mortimer at al., 1984).

Little information is available on the benefit of PGF_2 alpha treatment for chronic post-partum endometritis in cycling cows. However, favourable results have been obtained in clincial practice (Coulson, 1978, Ott and Gustafsson, 1981). This beneficial effect may be due to (a) induction of luteolysis if a functional CL is present, (b) a direct uterotonic effect, (c) stimulation of phagocytosis by uterine leucocytes (Razin et al., 1978) and, (d) stimulation of uterine defense mechanism consequent to a fall in circulating progesterone and a rise in circulating oestrogen (Paisley et al., 1986). Accordingly, PGF_2 alpha has been of some value in treating post-partum metritis in both cycling and non-cycling cows (Steffan et al., 1984). However, rate of recovery with this treatment is similar to other treatments like intrauterine Lugol's iodine infusion, I.M. oestrogen, I.M. oxytocin, and combination of these drugs (Paisley et al., 1986; Callahan & Horstman, 1987). Nevertheless, it can be considered as an alternative to treating individual cows with post-partum metritis because it is easy to administer and requires no milk withdrawal time after treatment (Paisley et al., 1986; Wichtel, 1991). PGF_2 alpha administered to cows once or twice between 14 to 40 days post-partum increases the first service conception rate or reduces the calving to conception interval in herds with poor reproductive efficiency or a high incidence of post-partum uterine infections (Young et al., 1984; Young & Anderson, 1986, Etherington et al., 1984, 1988; McClary et al., 1989). However, this beneficial effect is not manifested

during early post-partum period (Gross et al., 1986; Archbald et al., 1990) but in early post-partum cycling cow this treatment is very effective for anoestrus and pyometra associated with CL or luteal cyst (Seguin, 1980). In fact cows ovulating early in the post-partum period are more prone to develop pyometra (Olson et al., 1984) and prevention of early CL formation or induction of premature luteolysis are effective both prophylactically and therapeutically for post-partum uterine infections (Ott and Gustafsson, 1981; Olson et al., 1987).

Treatment with PGF_2 alpha is a good alternative to hysterectomy for canine pyometra complex (Burke, 1977; Johnston, 1979; Sokolowski, 1980). It causes luteolysis, cervical dilation and myometrial contraction in bitches with closed cervix pyometra and is also effective in evacuating uterus in open pyometra or endometritis (Lein, 1986; Nelson and Feldman, 1986). However, this treatment should be used with caution as the dog is unusally sensitive to PGF_2 alpha, the LD_{50} being 5.13 mg/kg (Sokolowski and Geng, 1977). Accordinlgy, in the bitch the dose range used has varied from 0.02 to 1.0 mg/kg (Johnston, 1979; Burke, 1982; Paradis et al., 1983; Lein, 1986; Nelson & Feldman, 1986). Some common treatment schedule include two injections of 0.1 mg/kg s.c. 24 hr apart (Johnston, 1979), 0.25 mg/kg s.c. daily for 5 days (Nelson & Feldanm, 1986) and multiple dosages of 0.025 to 0.25 mg/kg every 12 hrs depending upon clinical condition and side effects (Lein, 1986). With multiple injection schedule, treatment should begin with lower dose to minimize side effects which occur within20 to 120 minutes after injection and include hypersalivation, vomiting, diarrhoea, ataxia, urination, anxiety, depression and pupillary dilation followed by constriction (Lein, 1986). Simultanenously, parenteral broad spectrum antibiotic therapy is recommended for 1 (Nelson and Feldman, 1986) to 4 (Johnston, 1979) weeks depending upon the severity of the condition. Synthetic PGF_2 alpha analogues are not used in the bitch (Nelson & Feldman, 1986) and death from lethal dose occurs between 2 to 12 hrs after injection (Sokolowski, & Geng, 1977). This treatment is not recommended for older bitches (>8 years) or in severely ill bitches as clinical response may not be observed for 48 hr after beginning of therapy (Nelson & Feldman, 1986).

vii. Other applications:

Prostaglandin F_2 alpha has been employed for the termination of prolonged dioestrus in mare (Allen and Rossdale, 1973; Kenney et al., 1975; Allen, 1981) and is much more effective than intrauterine saline infusion (Arthur, 1970; Neely et al., 1975). However, this treatment is ineffective in mares which resorb or abort their conceptus after the 40th day of gestation until upto day 140 till the PMSG disappears from circulation. Additionally, ovarian cyst in cattle can be successfully treated with PGF_2 alpha either alone or in combination with Gn-RH or hCG (Kessler et al., 1978, Nakao et al., 1973). Also, PGF_2 alpha administration on day 9 after hCG or Gn-RH treatment of cystic ovary reduces the interval from initial treatment to the first oestrus from nearly 3 to 4 weeks to about 12 days without affecting fertility (Youngquist et al., 1986). In embryo transfer studies, PGF_2 alpha or its analogues increase the flexibility of timing superovaluation in donors treated with PMSG or FSH because superovulatory treatment can be initiated any time after day 6 of the cycle. This treatment also produces large number of normal embryos and the optimal time for treatment is between 8 and 12 day of the cycle (Betteridge, 1977). Furthermore, synchronization of oestrus in recipients is also achieved with PGF_2 alpha as synchronization between the stage of embryos and the reproductive tract of recipients is necessary for successful embryo transfer (Sreenan *et al.*, 1975).

In conclusion PGF_2 alpha is a potent 'local hormone' which affects a variety of physiological functions. However, its major practical application for controlled breeding of cattle is based primarily on its luteolytic and to some extent on its smooth muscle stimulating properties. However, optimum results are expected only under ideal management conditions as therapeutic application of PGF_2 alpha is not a substitute for good management.

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Preliminary Trials Of Multiple Ovulation And Embryo Transfer In Cows Under Field Conditions

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To effect rapid enhancement in genetic gain,Multiple Ovulation and Embryo Transfer (MOET) is recognised as the most effective technique and is used commercially at the field level,the world over.In India however, MOET in bovines found it's advent recently, and the first calf was born in 1986 (Ramkrishna and Bose, 1987). In an effort to make trials in the field, MOET was applied to crossbred cows (Bos taurus x Bos indicns) belonging to the farmers of Kaira district in Gujarat. Transfers with both fresh and frozen embryos were attempted with encouraging success.

Materials and Methods

In nine crossbred cows (5 HF & Jersey cross) a functional corpus luteum was palated on day 9-11 post-estrus (estrus=day 0) and same day supervoulation (SOV) treatment was given with an i.m. injection of Pregnant Mare Serum Gonadotrophin (PMSG; Folligon; Intercare). HF and Jersey crosses received 3000 & 2500 I.U. respectively. 30 mg Lupristiol (Prosolvin; Intervet; Holland) was injected i.m. at 48 & 60 hours after PMSG injection in two equal doses. Donors were inseminated three times using progeny tested bull's frozen semen, first at 48 hours after first PG inj., followed by 2 more A.I. at 12 hourly interval. Non-surgical embryo collection, evaluation, freezing-thawing and transfers were made as reported by Misra et.al. (1990). A portable freezer (R-206, Planer Biomad,UK) was carried to the field.Grade I embryos were selected and were frozen in 1.4 molar glycerol with -7^0C as the seeding-temperature. Crossbred (HF & Jer.) animals were selected as recipients and their estrus was synchronized with a single i.m. inj. of Lupristiol (15 mg) given at 36 hours after the PMSG inj. to the donor. For frozen embryo transfers either natural heat was considered for synchrony or the estrus was induced with 1 or 2 inj. of Lupristiol.Pregnancies were confirmed by rectal palpation 90 days after transfer.

Results and Discussion

The data related to the number of corpus lutem (CL),anovulatory follicles (FL), total and viable embryos recovered and unfertilized ovum (UFO) are shown breedwise in (Table 1).

Table :1 - **Superovulatory response and Embryo recovery in field cows**

Donor Breed	n	PMSG dose	CL	FL	Embryos recovered	viable	UFO
HF cross	5	3000 IU	15	8	7	4	1
		(x	3.0	1.6	1.4	0.8	0.2)
Jersey cross	4	2500 IU	32	8	25	8	12
		(x	8.0	2.0	6.2	2.0	3.0)
Total	9		47	16	32	12	13
		(x	5.2	1.8	3.5	1.3	1.4)

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Data show that ovulation rate in Jersey crossbreds (x=8.0) was higher than the HF crosses (x=3.0). Total and viable embryo recovery was better in Jersey crosses (x=6.0 ;2.0) as compared to HF crosses (x=1.4 ;0.8).

Seven fresh and twentytwo frozen embryos were transferred to synchronized recepients,resulting in three and six pregnancies giving 43 and 27% conception-rate respectively.

Healthy calves both by fresh (n=1) and frozen (n=5) embryo transfers are already born, while others are expected to calve. The embryo recovery by non-surgical techniques is reported to be ranging from 38% (Brand,1978) to 78% (Greve,1978).In our study, embryo recovery of 68% is comparable to that obtained by the authors under farm conditions (68% - unpublished data, 1989). Among the breeds, Jersey crosses have shown better performance than HF in terms of SOV-response, total and viable embryos.

Better SOV-response with FSH over PMSG has been widely acknowledged (Elsden, 1978; Crister, 1980; Grevel, 1983). In this study also the response is less compared to the farm results where FSH was profoundly used. The poor recovery of viable embryos also is attributed to the use of PMSG. In addition, one donor developed high fever during SOV treatment and yielded 10 UFO. Pregnancy-rate of 75% in the field work is reported by Ishii et al. (1989). Our results, although less in number and less impressive (43 and 27% pregnancy rate), are indeed inspiring and promise successful dissemination of MOET at the grassroot level.

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Superovulation With FSH-P And PMSG Hormones In Crossbred Cows And Heifers.

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ABSTRACT

Fourteen animals(8 cows and 6 heifers) were superovulated on day 11 of estrus with FSH-P or PMSG.In first group, eight I.M injections of 32 mg FSH-P were given daily for four days in descending doses.In second group PMSG was given I.M @2500 IU.50 mg Dinofertin I.M was given in both the groups (30 mg in the morning + 20 mg in the evening) for luteolysis on day 3 of initiation of superovulatory treatment.1200 IU HCG was given I/M at the time of A.I.No significant difference was observed in the onset or length of estrus in either group.All experimental animals showed intense estrus. The number of CL and anovulatory follicles was 10.57± 1.90 and 2.70 ±0.42, in FSH-P group and 10.42 ± 2.37 and 4.0 ± 0.30, in PMSG group respectively. The average number of CL and anovulatory follicles in cows (14.12 ± 1.46 and 3.25 ± 0.45) was significantly higher ($P<0.01$) than (5.66 ± 1.02 and 3.50 ± 0.42) in heifers.

* * *

PMSG, possessing both LH and FSH like properties, has been extensively used as a stimulator to follicle development, but presence of PMSG in the blood circulation after the time of ovulation, might have an adverse effect particularly on quality of developing embryos. Hence, alternatively pituitary Gonadotrophin was used for superovulation. Some workers have reported relatively poor response as well as wider individual variability with PMSG as compared to FSH (Monniaux *et al.*,1983, Elsden *et al.*, 1978), while Christer et al. (1980) found no difference between the response of the two gonadotropins. Hence this experiment was designed to study superovulatory response and estrus behviour with FSH-P and PMSG +HCG regimen, in pubertal and parous crossbred cows.

Materials and Methods

Fourteen crossbred animals (Jersey x Sahiwal) showing regular cyclic activity,with active corpus luteum (CL) and no signs of ovarian or uterine pathology were selected and randomly divided into two superovulatory treatment (A & B) groups. Group A comprising of 4 cows and 3 heifers was superovulated on day 11 of estrous cycle and received 32 mg of FSH-P (Schering Corporation.USA) in descending dose,twice daily for 4 days (2x5.5mg,2x4.5mg,2x3.5mg, 2x2.5mg,).Group B comprising 4 cows and 3 heifers was treated with 2,500 I.U.Folligon (PMSG: Intervet, Boxmer, Holland) I/M on day 11 of estrous cycle. 50 mg of Dinofertin was given I/M to both the groups (30mg in morning and 20mg in evening) on day 3 of initiation of superovulatory treatment. On day 5, 1500 I.U.Chorulon (HCG: Chorulon, Intervet, Boxmer, Holland) was given I/M. The animals were closely observed for estrus by parading vasectomised bull in the byre at 6 hourly interval and studied for dura-

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tion required for onset of estrus, length of estrus, behavioural symptoms and intensity of estrus as per score card (Singh and Kharche,1985). The superovulatory response was studied from the number of corpora lutea palpable per rectum on day 7 of treatment.

Results and Discussion

All the experimental animals (100%) exhibited estrus on superovulatory treatment. The response in FSH-P treated cows in the present study was superior than the reports of Rajamahendra *et al.*, (1987). The duration for estrus induction in FSH-P treated group was 43.00 ± 3.00 hrs.in cows and 46.01 ± 1.50 hrs.in heifers. The duration in PMSG + HCG group was 46.0 ± 2.0 hrs.in cows and 48.66 ± 0.66 hrs.in heifers. The average duration for onset of oestrus in both FSH-P and PMSG + HCG treated group was 44.28 ± 1.4 and 47.14 ± 1.22 hrs. respectively. However, the difference was not significant. These observations are in agreement with Scully *et al.*, (1982) and Yadav *et al.*, (1986). The duration for onset of estrus in heifers of both groups was slightly longer than cows, with non significant difference. The length of estrus with FSH-P regimen was 35.01 ± 2.38 and 26.0 ± 2.0 hrs. in cows and heifers, respectively, with an average of 31.14 ± 2.34 hrs. Whereas, the duration in PMSG + HCG treated group was 36.0 ± 2.16 and 32.66 ± 4.37 hrs. in cows and heifers, respectively, with an average of 34.57 ± 0.80 hrs. The length of estrus in cows of both groups was more as compared to heifers, although the difference was not significant. In both treatment groups, intense oestrus was observed with score ranging from 44 to 50.

The overall superovulatory response in terms of number of CL and anovulatory follicles was 10.57 ± 1.90 and 2.70 ± 0.42 in FSH-P treated animals and 10.42 ± 2.37 and 4.0 ± 0.30,respectively, in PMSG + HCG treatment group,which was almost similar between groups. These findings are in agreement with those of Christer et al.,(1980). The superovulatory response in FSH-P treatment observed in the present study was slightly better than Totey *et al.* (1987) and Totey *et al.*, (1988), but are comparatively less than Chupin, (1985). The superovulatory response observed in PMSG+HCG group is in agreement with Bhattacharya et al., (1987) and better than that (4.4 ± 4.9) reported by Laster *et al.*, (1973), but poorer than Scully *et al.*, (1982). The overall number of C.L. and anovulatory follicles observed in cows was 14.12 ± 1.46 and 3.25 ± 0.45 as compared to 5.66 ± 1.02 and 3.50 ± 0.42 in heifers. The superovulatory response in cows was significantly ($P<0.01$) higher than in heifers. Present findings differ from those of Moore (1975), who reported equal response in heifers and cows. It is concluded that the superovulatory treatment with FSH-P and PMSG regimen showed similar response in terms of duration for induction, length and intensity of estrus, C.L. number and anovulatory follicles palpated on day 7 of treatment, significantly better in crossbred cows than heifers. All the superovulated animals exhibited intense estrus behaviour.

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Synchronization Of Estrous In Surti Buffaloes

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ABSTRACT

Prostaglandin (Prosolvin, Intervet, Holland) treatment effect (n=85) on 25 Surti buffaloes with palpable corpus luteum (CL) on their return-to-estrus was analysed. 72% of the treatments resulted in standing estrus (within 136 hr) with a mean of 80.787± 3.84 hr.The difference between the observed and expected frequencies,assuming the hours in which the 61 animals came in estrus were normally distributed,was not significant ($p < 0.05$).

* * *

This experiment was done to find out the percentage of buffaloes (with more than 5 day old palpable CL) responding to prostaglandin (PG) treatment and the time they take to return-to-estrus,for deciding upon the number of recipients to be treated with PG for every buffalo superovulated and flushed.

Materials and Methods

25 non-lactating Surti Buffaloes being used as recipients for our embryo transfer programme from September to March (cool season) were analysed on their response to PG. These animals weighing between 300 to 400 kg were fed on green and dry fodder supplemented with By-pass protein feed. Urea molasses lick and water were given ad lib. They were sprayed with water at 11 am and 3 pm, if the day temp. exceeded 30^0C.

After checking the presence of a palpable CL (> day 5), luteolysis was induced with

single I.M. injection of 15 mg Luprostiol (n=85). Heat detection was carried out by parading a vasectomised bull (with a bull apron put on) in a paddock containing 15 - 20 buffaloes for 30 mins., three times daily at 6 am, 2pm and 10pm. A buffalo was said to be in standing heat, when she accepted the teaser bull / her mates.

Results and Discussion

72% PG treatments resulted in estrus within 136 hr of the injection. The mean time in which the buffaloes came in standing heat was 80.8± 3.84 hr. The frequency graph (Fig.1) shows that the number of animals which came in estrus at various 8 hour interval post PG inj. very nearly falls in a normal curve. The average time duration for which the standing heat was exhibited in the 61 observations, is given in Table 1.

Table 1 - **Average duration of standing estrus**

Estrus for (hrs)	no. of observations
8	10
16	17
24	22
32	5
40	2
48	2
56	1
64	1
72	1

Our 8 - hour interval heat detection programme using a teaser bull, spotted buffaloes with standing heat symptoms. Janakiraman and Mehta (1988) gave more emphasis to the secondary signs such as frequent micturition, vulval swelling, veginal discharge and bellowing, which are very useful under field conditions, where a farmer has one or two buffaloes kept tied and stall fed. Singh *et al.* (1984) found that vaginal discharge was the best indicator of estrus, whereas, Williams *et al.* (1986) used a pedometer to detect estrus in Egyptian buffaloes.

PGF_2 alpha, Cloprostenol, MGA+ oestradiol benzoate, Progesterone Releasing Intravaginal Device (PRID) and Synchromate-B with Luprostiol have all been used successfully in synchronisation of estrus in buffaloes with the return-to-estrus varying from 33.3% with Henniawati et al., (1985) to 100% with Kumar (1983) and Pargaonkar *et al.*, (1988). We could obtain 72% return-to-estrus. The higher success rate attained by Kumar (1983) and Pargaonkar *et al.* (1988) may have been due to the higher blood level of Progesterone. For synchroni-zation of estrus, only one injection was given by us for want of time and availability of recipient buffaloes.

Average time (80.8 ± 3.84 hr) the buffaloes took to return-to-estrus, was very much close to the results of Jindal *et al.* (1988), who got 77 ± 3.85 hr in lactating buffaloes and 75 ± 3.52 hr in buffalo heifers.

It is found that 80% of buffaloes which exhibited standing estrus, showed estrus symptoms for a period of 8 to 24 hrs (Av. 23.08 ± 1.68 hr). A few more buffaloes could have been found in standing estrus, had the heat detection been carried out more than three times a day.

This study indicates that (a) 72% of the PG treatments resulted in standing estrus within 24 - 136 hrs, with a mean of 80.8 ± 3.84 hrs, and (b) the average duration of estrus (standing heat) was 23.08 ± 1.68 hrs.

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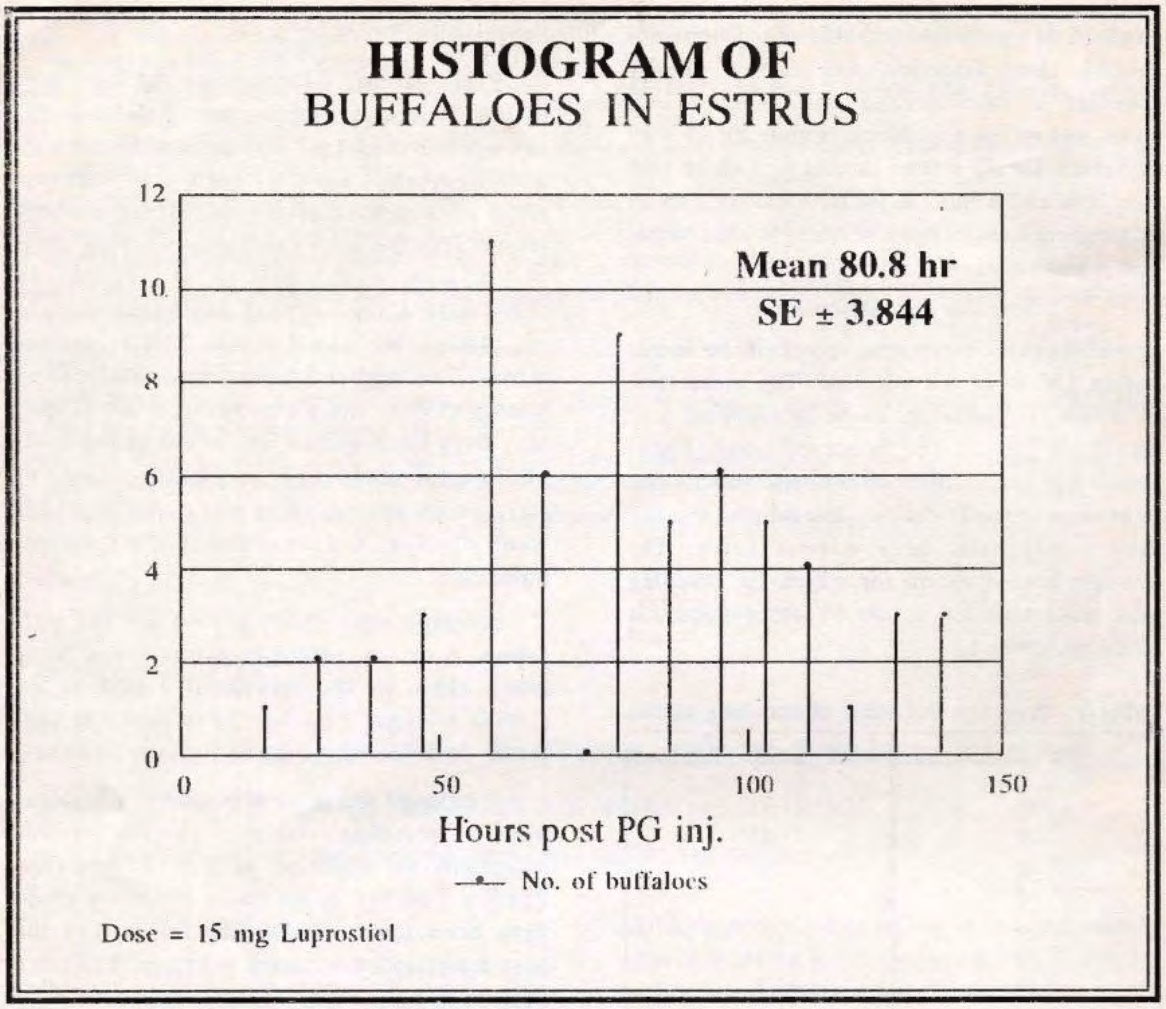

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Studies On Superovulatory Response And Surgical Embryo Recovery In Goats

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ABSTRACT

8 goats of Osmanabadi breed and its crosses with Beetal, Alpine and Saanen were selected as donors. All the donors were divided into 4 groups comprising of two donors in each group. Synchronization of oestrus in the donor does of group I and II was achieved by IM injection 12.5 mg. Progesterone (PG) per doe daily, for 16 and 14 days respoectively. In group III and IV oestrus was induced and synchronized with PMSG 100 I.U. subcut. Superovulation in donor does was achieved by PMSG 1000 I.U. subcut injection on last but one day of PG treatment in group I and II and same dose in group III and IV after induction of oestrus. In all the donor does hCG 1500 I.U. was injected I.V. after exhibition of oestrus. All the donors exhibiting oestrus were bred by fertile buck, 2 to 3 times during oestrus period. Embryos were recovered on day 5 post-breeding surgically, using DPBS with 10% heat inactivated goat serum. The overall mean number of intact G.F. and CL in donor does were 1.85 ± 0.06 and 9.42 ± 1.48. Mean percentage of ova recovery and fertilized ova was 7.42 ± 1.73 and 7.57 ± 1.37.

* * *

Importing exotic milch goat breeds for improving the milk production through A.I. is a time consuming process. Embryo Transfer Technology (ETT) in goats is a fast method of genetic improvement. In the present studies efforts were made to standardize superovulation and embryo recovery in goats.

Materials and Methods

Eight goats of Osmanabadi bred and its crosses with Beetal, Alpine and Saanen were selected as donors and divided into 4 groups of two donors each.

Donors of group I and II were injected 12.5 mg. Progesterone (Luteocycline Hindustan Ciba-Geigy Ltd., Bombay) I.M. for 16 and 14 days respectively. In group III and IV oestrus was induced by injecting PMSG 100 I.U. (Folligon Intervet International B.V.Boxmeer, Holland) subcut as a single dose.

For superovulation, PMSG 1000 I.U. was injected subcut in donors of group I and II on 15th and 13th day of PG treatment respectively. In group III and IV, PMSG 1000 I.U. was injected subcut just after induction of oestrus. hCG (Chorulon Intervet Internationl B.V.Boxmeer, Holland) 1500 I.U. was injected I.V. in donors of group I and II immediately after exhibition of oestrus and same dose was injected in donors of group III and IV 24 hrs after exhibition of oestrus. All the donors were mated with superior fertile buck three times duriug the oestrus period. Dulbecco's phosphate buffer saline (DPBS) containing 10% heat inactivated goat serum was used as flushing medium.

Embryo recovery was done surgically on day 5 post-oestrus, by performing midventral laparotomy under sedation and local infiltration. After exposure of genitalia, ovaries were observed for activity (GF/CL). During flushing of right uterine horn, polythene catheter was introduced in the fimbriated end of right

oviduct and Doyen's forceps was applied to prevent back flow of DPBS, 20 ml. DPBS was injected anterior to the forceps. Engorged uterine cornua was gently milked out to collect flushing in the petridishes applied at the tubal end. Same procedure was repeated for left uterine horn. Surgical wound was sutured and adequate post-operative care taken.

Petridishes with DPBS flushings were examined under a stereoscopic microscope at 25 x and 40 x magnifications.

Results and Discussion

Overall mean exhibition of oestrus in Progesterone (PG) and PMSG treated donors was 87.5%. These findings are higher than those reported by Agrawal (1986) in Barbari goats treated with MGA and PMSG (77.21%) and Doijode (1991) in goats treated with Progesterone, PMSG and hCG (75%).

Overall mean exhibition of oestrus in donors after PMSG injection was within 96 hrs (range 72 to 96 hrs). These findings are in agreement with those reported by Agrawal (1986) in Barbari goats treated with PG and PMSG and Doijode (1991) in Osmanabadi and crossbred goats treated with PG and PGF_2 alpha. However these are higher than those reported by Patil et al. (1984) in Angora goats treated with PG and PMSG (51.02 ± 4.66 hrs.) and Eiamvitayakoran et al. (1988) in goats treated with FSH and PGF_2 alpha (1.3 ± 0.04 and 1.2 ± 0.03 days).

Duration of oestrus in donor does was 24 to 36 hrs which is in normal range for goats.

Overall average number of intact follicles in donors was 1.85 ± 0.06. These findings are lower than those reported by Majumdar et al. (1990) in goats treated with FSH, PMSG and hCG (4.43) and Doijode (1991) in goats treated with PMSG and hCG (6.62 ± 1.34). This may be due to dose of hCG (1500 I.U.) used and proper timing of hCG administration.

Overall mean number of C.L. in donors was 9.42 ± 1.48. These findings are higher than those reported by Ott et al., (1979) in goats treated with PGF_2 alpha (2.1 ± 0.9) and Pandiya and Rathor (1986) in goats treated with PMSG + PGF_2 alpha (8.0) but are lower than reports of Armstrong et al. (1982) in Angora goats treated with PG and PMSG (10.5 ± 1.5) as well as PG and PGF_2 alpha (10.1 ± 3.0).

Overall mean percentage of ovulation to total ovarian activity in donors was 82.75 ± 2.12. These findings are higher than those reported by Doijode (1991) in goats treated with PMSG and hCG (34.29%).

Overall mean percentage of ova recovery in donors was 76.35 ± 7.8. These findings are in agreement with the reports of Ahmed and Maurya (1981) in goats treated with PMSG and hCG, but are considereably higher than those reported by Doijode (1991) in goats treated with PMSG and hCG (24.17 per cent). However, these findings are lower than those of Tervit et al. (1983) in goats treated with PMSG (82%) and Patil et al. (1984) in Angora goats treated with PMSG (100%).

Overall mean percentage of fertilized ova in donor does was 86.6 ± 5.0 per cent. These findings are in agreement with those reported by Tervit et al. (1983), but are higher than those reported by Bondioli and Wright (1981) in mixed bred goats treated with FSH and FSH + LH (67 and 50%) and Doijode (1991) in goats treated with PMSG and hCG (45.83%) and are lower than the reports of Patil et al. (1984) in Angora goats treated with PMSG (92%).

Difference in observations may be due to less number of animals in the present studies, variations in plane of nutrition, season, hormonal profile and breeds studied.

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Superovulation And Embryo Recovery In Black Bengal Goats

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Surgical method of embryo collection is used in small ruminants like sheep and goats. Hafez (1974) described the procedure for embryo collection through uterine and oviductal flushing. Gwatkin (1974) described the composition of Dulbeco Phosphate Buffer Saline (DPBS) solution suitable for flushing and storage of embryos. Tervit *et. al.* (1983) reported greater success in embryo collection in Angora goats. Indrajeet and Gupta (1987) reported variations in embryo recovery rates due to different days of collection after initiation of estrus.Detailed procedures for surgical collections of embryos have been reported by Jillela (1987). The present study was undertaken to compare the embryo recovery rate in Black Bengal goats as a result of embryo collection on different dates after appearance of estrus. The recovery rate was based upon the number of embryos collected out of the total number of follicles which ovulated.

* Part of thesis submitted to Birsa Agricultural University for award of M.V.Sc. degree

Material And Methods

12 adult Black Bengal goats with normal estrous activity were divided into 4 groups.Group I was control and received no treatment whereas animals under group II, III &IV were treated with PMSG 750 IU/goat/im on day 10 of the cycle, and 5mg PGF_2 alpha per goat i/m on day 11 of the cycle. HCG@ 500 IU per goat i/m was administered on the day of appearance of estrus.PMSG and HCG were obtained from InterCare Ltd, Calcutta and marketed respectively as Folligon & Chorulon. PGF_2 alpha marketed as Dinofertin was obtained from Alved products, Madras. All the goats were mated twice with a fertile buck on the appearance of estrus. The animals were maintained on 6 to 8 hrs, daily grazing and supplemental feeding of greens and concentrate allowed in addition. For the collection of embryos,the goats were subjected to mid-ventral laparotomy under local anesthesia. 2 ml. of chloropromazine was given I/m as a sedative. The embryos were collected after flushing the oviduct with a flushing fluid (DPBS). The embryos were collected on day 3 of estrus in the control group and on day 3, 4 and 6 of estrus respectively in groups II, III and IV. Post operative care was taken and all the animals recovered within two weeks.

Results and Discussion

In group I, an average of 2.6 ± 0.33 corpora lutea (C.L.) were observed whereas an average of 1.33± 0.33 embryos were recovered. The embryo recovery was found to increase to 57.14% (12 embryos out of 21 C.L.) in group-II and 64-51% (20 embryos out of 31 C.L.) in group III. The embryo recovery was found to decrease to 22.01% (8 embryos out of 36 C.L.) in group IV. It is thus obvious that maximum recovery was obtained in group III that is on the 4th day following appearance of estrus and only slightly lower on the 3rd day of collection. The values declined sharply when the collections were made on the 6th day following estrus. No significant difference was observed in embryo recovery between different treatment groups (Table -1).

Our findings are in agreement with the reports of Indrajeet and Gupta (1987) who reported highest recovery in Barbari goats on the 3rd and 4th day following estrus. Vadnere and Mani (1986) reported 60 to 75% recovery of embryos. Agrawal *et al* (1982) reported 61.90% recovery of embryo. Armstrong *et al* (1983) reported low recovery rates in Angora goats flushed later than day 5 after onset of estrus. Sugie *et al* (1980) reported that the ova resided for 3 to 4 days after estrus in the oviduct and thereafter migrated to the uterus. Poor recovery of embryos on day 6 following estrus may be due to the migration of embryos into the uterus.

Table-1: Effect of days on embryo recovery in goats

Group	Collection of embryo following appearance of estrus.	Total number of corpora lutea.	Total number of embryos collected.	Percentage of embryo recovery.
Group I (Control)	3rd day	8	4	50.00
Group II	3rd day	21	12	57.14
Group III	4th day	31	20	64.51
Group IV	6th day	36	8	22.01

3 goats were included in each group.

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Ova Recovery Rate In Relation To Various Doses Of HCG Treatment In Goat

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ABSTRACT

A dose rate of 750 IU of HCG could be very effectively used for harvesting the maximum number of ova in local goat of Assam.

* * *

Literature concerning the ova recovery rate after superovulation with FSH or PMSG with or without LH or its analogue in various species of animals though available, is not adequate in goat ; especially on the use of HCG. The present study reports the ova recovery rate in relation to various doses of HCG treatment in local goat of Assam.

Material and Method

A total of 20 sexually matured healthy local goats of Assam, 2-3 years old, were included in this study. The animals were maintained under semi-intensive system of rearing.

The animals were divided into 4 groups A,B,C and D of 5 animals each. The animals of group B, C and D received an I.M. injection of 550, 650 and 750 IU of HCG (Chorulon-Chorionic gonadotropin B. Vet. C(L.H.) Intervet, Holland at 6 hours post onset of oestrus, respectively; while the animals of group A

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served as control with an I.M. injection of distilled water at the same time schedule.

Laparotomy and collection of ova

Laparotomy was carried out at 48 hours after the onset of oestrus in all the animals of 4 groups as per routine surgical procedure. After laparotomy, the right fallopian tube was exteriorised carefully and after soaking with lukewarm sterile normal saline, a sterile plastic catheter was introduced through the infundibular end and other end of the catheter was kept over a petridish to collect the flushed out fluid. A blunt sterile needle was inserted through the uterotubal junction. The oviductal flushing was made for recovery of ova by pushing about 5 ml of normal saline towards the infundibular end with the help of a sterile syringe. Subsequently, the left fallopian tube was taken out and the oviductal flushing and collection of the fluid was done as in the case of right fallopian tube. Then both the fallopian tubes were placed in proper position after soaking with lukewarm sterile normal saline.

Immediately after the collection of oviductal fluid, detection and evaluation of ova was made under the binocular compound microscope.

Results and Discussion

Ova recovery rate was highest (2.40 ± 0.51) in the group treated with 750 IU of HCG and lowest (1.80 ± 0.49) in the group treated with 550 IU of HCG. The value (2.00) remained same in both control and in group that received 650 IU of HCG. Although difference existed in ova recovery rate among the groups of the experiment, it was not significant.

In terms of ovulation, the respective percentage of ova recovery rates were 100, 69.23, 90.91 and 85.71 in control, 550 IU, 650 IU and 750 IU HCG treated groups. There was a clear evidence of an inverse relation of ovulation rate to ova recovery rate. Similary evidence was reported by McGovern et al. (1969), who could recover only 2, 8, 3 and 2 eggs against 4, 23, 7 and 8 ovulations, respectively, from ewes treated with either HCG alone or with PMSG and HCG in combination.

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Plasma Progesterone Profiles In Superovulated And Oestrous Synchronized Goats.

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The blood plasma progesterone level is indicative of the activity of the corpus luteum and therefore estimation of blood progesterone may help in detection of superovulatory response and the extent of oestrous synchronization. The present investigation was therefore aimed at determining the plasma progesterone concentration in superovulated and oestrous synchronized goats.

Twelve adult nondescript goats of 2 to 4 years of age were used for the experiment and were divided in two equal groups at random. Group I goats were superovulated with 1000 IU of PMSG (Folligon, Intervet) in the luteal phase of the oestrous cycle followed by another 600 IU the next day alongwith 7.5 mg of PGF_2 alpha (Dinofertin, Alved laboratories, Madras) and were given 1500 IU HCG (Chorulon, Intervet) I.V. on the following day. Group II animals were estrous synchronised by PGF_2 alpha at the same dose level. Laparotomy was done in group I goats, 5 days after the start of oestrus and the number of corpora lutea were counted on both the ovaries. Progesterone estimation of blood serum from day 1 to 4 postestrus was done by R.I.A.

Results and Discussion

The mean blood plasma progesterone concentration in the superovulated and oestrous synchronized goats were found to be 5.07 ± 0.37 and 3.97 ± 0.09 ng/ml respectively, prior to treatment. Similar concentrations (5.0 ng/ml) were reported in pre-treatment goats by Mutiga and Baker (1984). The level of progesterone after treatment with PMSG and PGF_2 alpha dropped from 4.07 ± 0.29 and 4.09 ± 0.2 to 1.13 ± 0.84 and 1.53 ± 0.57 ng/ml respectively and remained at these levels for 2 days during oestrus. Jain and Madan (1986) recorded lower values of progesterone (0.09 and 0.23 ng/ml) in HCG non–treated and treated goats after PMSG and PG injections. The precipitous fall in the progesterone concentrations after PG injection could be due to the luteolysis of cyclic corpora lutea. There was an increase in the level of progesterone in both the groups on day 3 of PG injection. On day 5, the concentration of progesterone in superovulated group was 8.07 ± 0.79 ng/ml (range 7.0 to 9.6 ng/ml) which agreed with the observations of Mutiga and Baker (1984), who found the peak concentration of progesterone in superovulated goats as 10 to 35 ng/ml. The lower concentration of plasma progesterone (3.47 ± 0.37 ng/ml, range 3.0 to 4.2 ng/ml) on day 5 of PG injection in synchronised group, suggests the presence of fewer number of corpora lutea as compared to the superovulated group. In superovulated goats on day 6 of PG injection, the corpora lutea ranged from 9-13. A positive but non-significant correlation (r=0.52) was observed between the number of corpora lutea and progesterone concentration. The co-efficient of regression of progesterone concentration on the number of corpora lutea was found to be nonsignificant ($P< 0.05$). This was however in contrast to the findings of Indramani and Vadnere (1986) in an earlier study.

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A Note On Cryopreservation Of Caprine Embryos By Vitrification

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Preservation of mammalian embryos by vitrification is a new promising approach in the field of cryobiology (Rall and Fahy, 1985, Massip et al, 1987, Agrawal and Polge, 1989). Informations on preservation of caprine embryos by vitrification are meagre. Survival of vitrified caprine embryos using a combination of two cryoprotectants of low toxicity (glycerol and 1-2 propanediol) has been studied in the present experiment.

Vitrification method detailed by the author in his earlier communication (Agrawal and Polge, 1989) was used in this experiment. Seventeen morphologically normal 4- to 8-cell embryos (replicates) after flushing from superovulated donors and washing 2-3 times with Dulbecco's PBS enriched with 10% goat serum were exposed to intracellular cryoprotecting medium (glycerol 10%, 1-2 propanediol 20% in D-PBS) for 10 minutes. Embryos were then transferred to extracelluar vitrification medium (25% glycerol; 25% 1-2 propanediol in PBS). The temperature of cryo-protecting and vitrification media during embryo exposure was maintained at 4^0 C either in refrigerator or in an ice bucket. Two to three embryos in vitrification medium and diluent (1 M sucrose) in determined proportions were loaded in 0.25ml straw. The straw was plunged in LN_2 in two steps (i.e. the portion of the straw up to the level of embryos in vitrification medium plunged immediately and then rest of the portion progressively). After about a month, straws were taken out and thawing was done in a water bath maintained at 20°C. The contents of the straw were mixed by shaking, emptied in a petri-dish and left for 10 minutes at room temperature. Frozen- thawed embryos after three washings in culture medium (Dulbecco's PBS enriched with D-glucose, sodium pyruvate and 10% goat serum) were kept in CO_2 incubator (maintained at 38°C temp. and 95% RH in atmospheric air) for in-vitro development or transferred to suitable recipients for in-vivo development.

16 out of 17 embryos frozen by vitrification were recovered on thawing. No apparent morphological damage in zona pellucida and vitellus was observed in thawed embryos. Osmotic changes, viz swelling and shrinkage were however observed when embryos were treated with vitrification, sucrose diluent and isotonic media (Figs 1-3). Six out of 10 embryos (60%) cleaved once within 24 hrs of incubation. Out of five frozen-thawed embryos transferred to two recipients, one

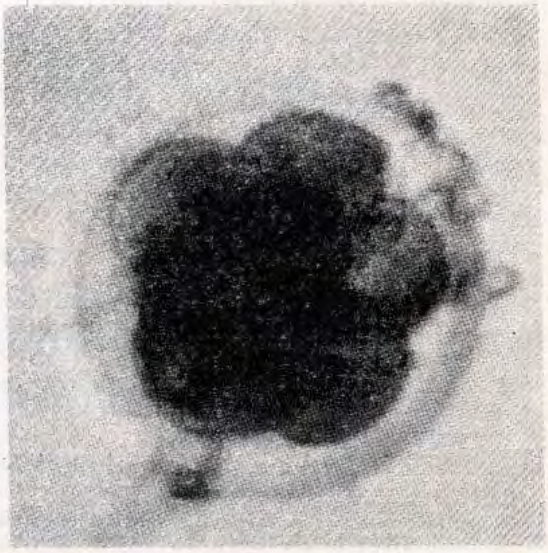

Fig.1. 8-C caprine embryo after flushing.

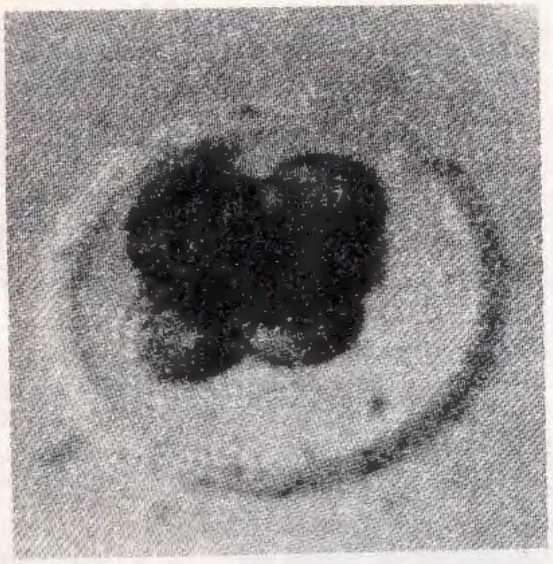

Fig.2. 8-C caprine embryo after transfer to vitrification medium.

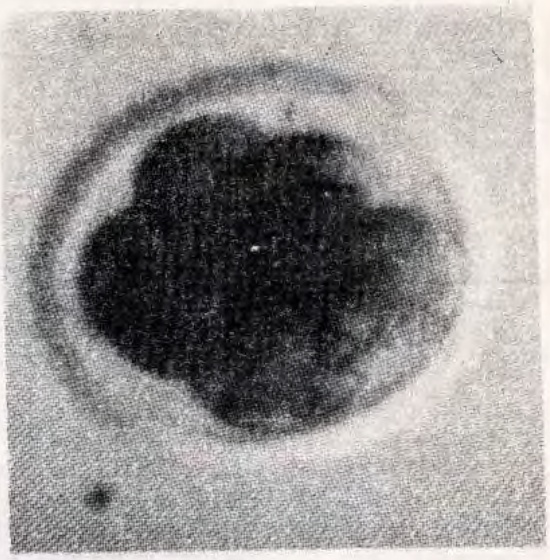

Fig.3. The frozen-thawed embryo (8-C) regains its normal volume after transfer into sucrose-free isotonic medium.

recipient exhibited oestrus within 21 days of transfer. Oestrus in other recipient was delayed. None of the embryo developed to full term (Table 1).

Agrawal and Polge (1989) obtained 20.23% survival of mouse embryos as compared to 60% survival of caprine embryo in the present experiment using the same freezing protocol except the temperature of cryoprotectant and vitrification media before and after embryo exposure was maintained at 4°C either in refrigerator or in an ice bucket. Rall and Fahy (1985) reported a survival rate as high as 90% on freezing mouse embryos by vitrification method. In their study exposure of mouse embryos to vitrification medium was done in a cold room (4°C) and embryos used were at late stages of development. Early cell stages of embryos (4-8C) which are more sensitive to cooling than late stages (Whittingham, 1971, Willadsen *et al*, 1976) were used in the present experiment. Though the temperature of cryoprotecting and vitrification media was maintained at 4°C, it was difficult to maintain this temperature during embryo processing/examination under the microscope which was done at a room temperature of 20°C. With cold room facilities and by using late stage of embryos, there is a possibility to improve embryo survivability in subsequent experiments. Massip *et al* (1987) reported a pregnancy rate of 39% in cows. The reasons for the failure of in-vivo development in one recipient and possible resolution of embryo/fetus in other recipient is difficult to explain at the moment and needs further investigation involving large number of embryos.

The author thanks Director of the Institute for providing necessary facilities.

Table 1 : **Freezing of caprine embryos by vitrification**

1.	No. of embryos frozen	17
2.	No. of embryos recovered on thawing	16
3.	No. of thawed embryos exhibited morphological damage	Nil
4.	No. of frozen-thawed embryos used for culture	10
5.	No. of embryos cleaved within 24 hrs of culture	6
6.	No. of frozen-thawed embryos transferred	5
7.	No. of embryos developed to full term	Nil

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Fertility Of Semen Frozen With Additives To Semen Diluent

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ABSTRACT

Frozen semen from three adult bulls each of Holstein-Fresian, Jersey and 50% Holstein-Fresian crosses was used for studying the effect of caffeine puris and surfactants added to the diluent prior to processing and freezing on fertility. Caffeine puris 7mM and 0.5% surfactant mixture of sodium and Triethanolamine lauryl sulphate was added to the Tris-Citric Acid-Fructose-Egg Yolk Glycerol diluent. Split samples of semen from each bull were diluted in two treatments and one control diluent. The treated frozen semen alongwith control was tested on 2,499 first AI in 5 different regions for fertility. Treatments and control did not differ significantly.

* * *

Recently caffeine puris (Gehlout and Srivastava. 1987: Miyamoto and Nishikawa, 1979; and Singh *et al.* 1986) and a mixture of

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sodium and triethanolamine lauryl sulphate (Arriola, 1982; Arriola and Foote, 1987; and Foote and Arriola, 1987) have been used as additives to semen diluents for improving post thaw motility and fertility of frozen bovine semen.

Bhosrekar *et al.* (1990) reported significant increase in post thaw motility as compared to control on use of surfactant to semen diluent. Further to this, fertility trials were undertaken which are reported hereunder.

Materials and Methods

Three adult bulls each of Holstein-Fresian. Jersey and 50% Holstein Fresian cross bred were selected for this study. All the bulls were in semen collection schedule for quite some time with optimum freezability and fertility of semen.

Tris-citric acid-fructose eggyolk glycerol having following composition was used as a semen diluent.

TRIS (Hydroxy methyl) amino methane	12.1 g
Citric acid	6.8 g
Fructose	5 g
Glycerol	32 ml
Sterile double glass distilled water	368 ml

The diluent was split in three portions. Portion 2 and 3 were added 7 mM caffeine puris and 0.5% mixture of sodium and Triethanolamine lauryl sulphate. The semen ejaculate from each bull after evaluation for initial motility, sperm concentration was split in three portions and was added to three different portions of diluents so as to keep 30 million sperms per dose, of 0.28ml diluted semen (Minitub). The processing was carried out at 22°C room temperature. The packaging of semen was done by automatic packaging machine (MT 65). The filled straws were then arranged horizontally on freezing racks and cooled to 5°C in refrigertor. After 6 hours equilibration period at 5°C, the semen straws were frozen by placing the racks on grill touching liquid nitrogen (LN_2) in a static vapour column in 320 MVE container. After 10 minutes the frozen semen straws were collected and placed in precooled goblets and immersed in LN_2.

The post-thaw evaluation for forward motility was done by thawing representative straw in warm water bath at 37°C for 30 seconds and examined under phase contrast microscope with biotherm. The frozen semen was sent to field cattle breeding centres in 5 different regions in Maharashtra State after one month quarantine period. The frozen semen was coded to avoid bias on the part of field AI technicians. The data on conceptions diagnosed by actual rectal palpation for 2,499 first AI from 3 Holstein Fresian, 3 Jersey and 3 crossbred bulls (HF 50%) was compiled and analysed statistically.

Results and Discussion

Data for the conception rate with standard errors., region wise and breed-wise for two treatments and one control group are presented in Tables 1 and 2.

Analysis of variance showed highly significant difference ($P<0.01$) between regions which was expected due to different agro-ecological conditions. No significant differences were observed between breeds as well as between treatments. However, slightly higher conception rate was recorded in case of crossbred and Jersey semen treated with caffeine puris and surfactants. Miyamoto and Nishikawa (1979), Singh *et al.* (1986), Gehlout and Srivastava (1987) recorded significantly higher motility and fertility for frozen semen pre-treated with caffeine citrate. Similarly, Arriola and Foote (1987) and Foote and Arriola (1987) reported significantly higher motility and fertility. (Non return rates) for frozen semen pretreated with surfactants 0.5%

mixture of sodium and Tiethanolamine lauryl sulphate in Tris-egg yolk diluent. However, they could not record any increase in fertility when surfactant mixture was added to whole milk egg yolk diluent. According to them surfactant mixture may be acting through extender buffer on sperm membranes perhaps making them somewhat porous and less sensitive to osmotic or glycerol shock during freezing and thawing.

Table 1 : Mean conception rate (%) with standard error for different treatments, region-wise

Treatment	Region 1	Region 2	Region 3	Region 4	Region 5
Treat 1 (control)	61.40 ± 6.505 (57)	80.26 ± 4.59 (76)	71.30 ± 2.52 (324)	72.53 ± 4.70 (91)	66.41 ± 2.95 (256)
Treat 2 (caffeine puris)	50.94 ± 6.93 (53)	84.21 ± 3.43 (114)	71.31 ± 2.39 (359)	81.43 ± 4.68 (70)	57.81 ± 3.09 (256)
Treat 3 (Surfactant mixture)	56.25 ± 7.24 (48)	90.63 ± 2.99 (96)	71.39 ± 2.36 (367)	83.15 ± 3.99 (89)	64.2 ± 3.08 (243)

Table 2 : Mean conception rate (%) with standard deviation breed-wise.

Item	Cross bred	HF	Jersey	Overall
Treatment 1 (control)	64.89 ± 4.18 (131)	75.57 + 2.16 (397)	64.49 + 2.88 (276)	70.02 + 1.62 (804)
Treatment 2 (caffeine puris)	71.76 ± 3.94 (131)	68.79 + 2.21 (439)	66.67 + 2.81 (282)	68.54 + 1.59 (852)
Treatment 3 (Surfactant mixture)	75.23 ± 4.14 (109)	73.67 + 2.12 (433)	68.11 + 2.69 (301)	71.89 + 1.55 (843)

Figures in parentheses are number of inseminations on which conception rates are calculated.

Table 3 : Analysis of variance.

Source of variance	Degrees of freedom	Mean sum of squares.
Region	4	37498.367 **
Breed	2	6196.416 NS
Treatment	2	2456.842 NS
Residual	2490	2031.686

** Significantly different at 1% probability level.
NS Not significant.

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Efficacy Of Certain Extenders On Leakage Of Phosphatases From Buffalo Spermatozoa During Deep-Freezing

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ABSTRACT

Egg-yolk citrate glycerol (EYCG), Tris yolk glycerol (TYG) and Citric acid whey glycerol (CAWG) extenders were compared for the leakage of Alkaline and Acid phosphatase enzymes from buffalo spermatozoa during different stages of deep-freezing. A total of 40 ejaculates from five buffalo bulls (8 per bull) exhibiting more than 70% initial motility, were frozen in these extenders. Enzyme levels were studied in the seminal plasma during prefreezing stages (fresh semen, after complete extension and after equilibration period) and post-freezing stages (24 hours and 7 days after freezing). It was found that the leakage of Alkaline and Acid phosphatases was least in TYG and most critical stage of Freezing was between equilibration and 24 hours after freezing.

* * *

The efficacy of extenders used for deep-freezing of buffalo semen was mostly tested by post-thaw motility apart from fertility trials. Any trauma to sperm membrane due to cold shock, ice cyrstals disruption or unfavourable concentration of extender's ingredients causes extracellular release of enzymes (Tuli *et al.*, 1982). Acid and Alkaline phosphatases are the most active and best known dephosphorylating enzymes. Keeping in view the involvement of these enzymes in sperm metabolism, an effort was made to study their leakage under varied conditions of processing and freezing semen in three extenders: Egg yolk citrate

glycerol (EYCG), Tris yolk glycerol (TYG) and Citric acid whey glycerol (CAWG).

Materials and Methods

A total of 40 ejaculates from 5 Murrah buffalo bulls (8 per bull) were collected at weekly intervals. Each ejaculate was subjected to deep-freezing. Three extenders, namely Egg yolk citrate glycerol (EYCG), Tris yolk glycerol (TYG) and Citric acid whey glycerol (CAWG) were used. Penicillin G Sodium (1000 I.U./ml) and Streptomycin Sulphate (1 mg/ml) were added to all the extenders. The pH of all extenders was ajusted to 6.8. The final concentration of glycerol was 7% by volume. Each ejaculate was divided into four fractions. Of these, three fractions were diluted separately in the three extenders used. The dilution rate was calculated on the basis of sperm concentration in order to have 40 million spermatozoa in 0.5 ml diluted semen. The semen was deep-frozen (Cassou, 1976) with 4 hr. equilibration period.

The level of alkaline phosphatase (AKP) and acid phosphatase (ACP) enzymes in the seminal plasma was measured in pre-freezing stages : stage 1 (fresh semen), stage 2 (after complete extension), stage 3 (after equilibration period and post-freezing stages), stage 4 (24 hours after freezing) and stage 5 (7 days after freezing). To study enzyme levels in seminal plasma of stage 1 (fresh semen), one fraction of each ejaculate was diluted in normal saline (as per dilution rate). One ml of semen, at stage 1, stage 2 and stage 3 of each extender was centrifuged at 3000 r.p.m. (g = 1300) for 15 minutes at room temperature and supernatant (seminal plasma) was used for enzyme assay. In the same way, at stage 4 and stage 5, two straws from each extended semen were thawed at 37°C for 1 min, centrifuged and supernatant used for enzyme assay. AKP and ACP enzymes were estimated by the method of Wootton (1964). The data were analysed by using F test (Snedecor and Cochran, 1967).

Results and Discussion

The mean AKP and ACP concentrations in the seminal plasma at various stages of deep-freezing in different extenders are furnished in Table 1.

Enzyme levels in pre-freezing stages: The mean AKP and ACP levels in fresh semen were recorded as 28.06 ± 0.78 and 2.34 ± 0.13 n. mol phenol liberated/min/ml. However, slight differences in the values were reported by Chauhan and Srivastava (1973) and Choudhary and Gangwar (1977). This disparity might be attributed to the differences in breed, age, environmental factors and possible variations in contributions from epididymis and accessory sex glands. There was non-significant difference between the extenders at all pre-freezing stages and between the stages in all the extenders.

Enzyme levels in post-freezing stages: The mean AKP level in the seminal plasma at stage 4 of deep-freezing was found to be 76.02 ± 5.90 in EYCG; 61.94 ± 4.00 in TYG and 95.72 ± 7.22 units in CAWG. The resepective values of ACP were recorded as 7.08 ± 0.47, 4.48 ± 0.32 and 8.04 ± 0.82 units, respectively. The levels of AKP in the seminal plasma at stage 5 were 137.48 ± 9.11; 92.18 ± 9.70 and 152.44 ± 4.78 units in EYCG, TYG and CAWG extenders, respectively. The respective values for ACP were 9.84 ± 0.70, 6.55 ± 0.39 and 11.97 ± 1.00 units.

In the present study the AKP and ACP levels were significantly higher ($P<0.01$) in seminal plasma at stage 4 than stage 3 in all the three extenders. This could be attribnted to temperature shock and sperm cell injury associated with freezing (Crabo *et al.*, 1971) rupture of sperm cell mebrane or increase in cell membrane permeability without rupture (Roychoudhary *et al.*, 1974) causing the

leakage of intracellular enzymes into the extra cellular fluid.

It has also been recorded that AKP and ACP levels were significantly higher (P<0.01) at stage 5 in comparison with stage 4 in all the three extenders which suggested further leakage of AKP and ACP into extracellular fluid during storage and this might be due to increase in the concentration of ageing and decaying spermatozoa in cryopreserved semen (Mann and Lutwak-Mann, 1981).

It has been found that significantly (P<0.01) lower levels of AKP and ACP in seminal plasma were observed in TYG than in EYCG and CAWG at stages 4 and 5, which could be due to maximum protective action of Tris to sperm membrane against cellular injury (Crabo et al., 1971) as compared to EYCG and CAWG extenders. On the basis of AKP and ACP leakage, it is concluded that TYG is the most suitable extender out of three extenders used for deep-freezing of buffalo semen. The most critical stage of freezing is between equilibration and 24 hours after freezing where maximal leakage of enzymes was recorded.

Table 1: **Mean levels of Alkaline and Acid phosphatases in seminal plasma of neat buffalo semen and during different stage of deep-frrezing in various extenders (n. mol. phenol liberated/min/ml).**

Stages of freezing	EYCG		TYG		CAWG	
	Alkaline phospha-tase (AKP)	Acid Phos-phatase (ACP)	Alkaline phospha-tase (AKP)	Acid Phos-phatase (ACP)	Alkaline phospha-tase (AKP)	Acid Phos-phatase (ACP)
Fresh seminal plasma (stage-1)	28.06^{a} ± 0.78	2.34^{1} ± 0.13	28.06 ± 0.78	2.34 ± 0.13	28.06 ± 0.78	2.34 ± 0.13
After complete extension (stage-2)	30.02^{b} ± 0.69	3.06^{2} ± 0.46	33.08^{f} ± 2.95	2.40^{6} ± 0.15	32.62^{j} ± 1.10	2.48^{10} ± 0.16
After equilibration period (stage-3)	30.92^{c} ± 2.14	2.27^{3} ± 0.09	32.96^{g} ± 2.08	2.41^{7} ± 0.13	31.20^{k} ± 1.78	2.76^{11} ± 0.09
24 hrs after freezing (stage-4)	76.02^{d} ± 5.90	7.08^{4} ± 0.47	61.94^{h} ± 4.00	4.48^{8} ± 0.32	95.72^{l} ± 7.22	8.04^{12} ± 0.82
7 days after freezing (stage-5)	137.48^{e} ± 9.11	9.84^{5} ± 0.70	92.18^{i} ± 9.70	6.55^{9} ± 0.39	152.44^{m} ± 4.78	11.97^{13} ± 1.00

Analysis for AKP

Between extenders : d<l, h<l, i<n, i<m, (P<0.01). h<d, e<m (P<0.05).

Between stages : c<d, d<a, g<h, h<i, k<l, l<m, (P<0.01). CD = 18.40 (P<0.01), CD = 13.81 (P<0.05).

Analysis for ACP

Between extenders : 8<4, 8<12, 9<5, 9<13 5<13 (P<0.01)

Between stages : 3<4, 4<5, 7<8, 8<9, 11<12, 12<13, (P<0.01). CD = 1.66 (P<0.01).

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Study On Freezing Of Goat Semen In Skim Milk Extender With Different Glycerol Levels and Equilibration Periods*

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ABSTRACT

A total of 20 ejaculates comprising 4 from each of 5 bucks were used to study the effect of 5, 6 and 7 % glycerol in skim milk extender and 2, 4 and 6 hrs. of equilibration on quality of frozen semen by split sample technique. The semen was washed twice prior to extension and vapour frozen in French straws (0.5 ml). The sperm motility and percentage of intact acrosomes were studied after equilibration and freezing. There was no significant effect of glycerol levels, equilibration periods and their interaction on sperm motility and percentage of intact actosomes after equilibration and freezing, except on sperm motility after freezing which was significantly ($P<0.05$) affected by equilibration periods. The sperm motility after freezing was significantly ($P<0.05$) higher in semen equilibrated for 4 hrs. The percentage of intact acrosomes decreased as the glycerol level in the extender and equilibration period increased.

Skim milk extender with combination of 6% glycerol and 4 hrs equilibration resulted in better quality frozen semen.

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The optimum glycerol level and equilibration period differ in different extenders (Andersen, 1969; Sahni and Roy, 1972; Deka and Rao, 1986). The present study was aimed to determine the effect of glycerol level and

* art of the M.V.Sc. thesis of the first author submitted to Assam Agricultural University, Khanapara

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equilibration period in skim milk extender on sperm motility and acrosomal integrity of frozen buck semen.

Materials and Methods

20 ejaculates comprising 4 from each of 5 bucks (4 Beetal and 1 crossbred) collected using A.V. were used in this study. If a buck donated less than 0.9 ml of semen, a second ejaculate was collected and pooled with the first. Immediatley after collection the ejaculate was washed twice using sodium citrate buffer (2.9%) as described by Deka and Rao (1984). The centrifugate was diluted @ 1:5 using non-glycerolated fraction of skim milk extender (Rajkonwar *et al.*, 1977) taking the ejaculate volume prior to washing into consideration. The primary extended semen was split into 3 parts to which glycerolated fractions (volume equal to non-glycerolated fraction) containing 10, 12 and 14% glycerol were added in 2 steps at 5°C. The semen was frozen in French straws (0.5 ml) after 2, 4 and 6 hrs equilibration at 5°C. The freezing and thawing of semen were done as per Deka and Rao (1984). The sperm-motility and acrosomal morphology were studied by conventional method and Giemsa staining technique (Watson, 1975) respectively after equilibration and freezing. The statistical analysis of the data was made after transforming the percentages into angles (Snedecor and Cochran, 1967).

Results and Discussion

The mean sperm motility after equilibration was not significantly influenced by glycerol levels, equilibration periods and their interaction. This supports the observation of Deka and Rao (1986). The sperm motility after freezing differed significantly ($P<0.05$) between equilibration periods but not between glycerol levels and due to interaction. Similarly, Fraser (1962) did not record marked variation in post thaw motility when glycerol level in skim milk extender varied from 6 to 9%. In contrast, Sahni and Roy (1972) recorded significantly higher post freezing motility with 6% glycerol than with 3 and 9% glycerol in cow milk extender. In the present study, although not significant, the sperm motility after freezing was apparently higher with 6% glycerol than with 5 and 7% glycerol (Table 1). On critical difference test, it was found that mean sperm motility after freezing was significantly ($P<0.05$) higher in semen equilibrated for 4 hrs than in that equilibrated for 2 hr. This is in agreement with the observations of Deka and Rao (1986) and Sinha *et al.*, (1987) in Tris extender. On the contrary, in skim milk extender higher post thawing sperm motility was reported with equilibration period of 1 hr. (Andersen, 1969) and 8-24 hrs. (Fraser, 1962). These variations could be attributed to the differences in composition of the extender and methods of processing.

There was no significant effect of glycerol level, equilibration period and the interaction on the percentage of intact acrosome after equilibration and freezing. This is in agreement with the observations of Wiggin and Almquist (1975) in skim milk extender in bull semen. In this study, it was observed that the percentage of intact acrosomes decreased as the glycerol level and the equilibration period increased from 5 to 7% and 2 to 6 hrs. respectively (Table 1). This supports the observations of Deka and Rao (1986).

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Table 1 : **Sperm motility and percentage of intact acrosomes (Mean* ±SE) after equilibration (E) and after freezing (F) in skim milk extender with different glycerol levels and equilibration periods.**

Equi-libration periods	Sperm motility (%) N=60								Intact acrosomes (%) N=60							
	5% glycerol		6% glycerol		7% glycerol		Overall Mean		5% glycerol		6% glycerol		7% glycerol		Overall Mean	
	E	F	E	F	E	F	E	F	E	F	E	F	E	F	E	F
2 hours	71.57 ±1.18	39.40 ± 4.17	72.90 ± 1.24	46.65 ± 4.40	72.68 ± 1.35	41.42 ± 4.29	72.39 ± 0.74	43.04[b] ± 2.25	65.53 ± 2.93	56.37 ± 2.41	65.38 ± 2.04	56.30 ± 2.80	65.15 ± 1.91	54.91 ± 2.92	65.37 ± 1.30	55.87 ± 1.50
4 hours	70.51 ± 1.21	50.12 ± 3.00	71.53 ± 1.18	52.65 ± 2.89	70.27 ± 1.28	49.48 ± 3.38	70.78 ± 0.72	50.75[a] ± 1.67	64.40 ± 2.46	56.06 ± 1.85	63.28 ± 2.61	54.72 ± 2.66	61.96 ± 2.49	54.69 ± 2.74	63.21 ± 1.42	55.16 ± 1.36
6 hours	70.33 ± 1.31	41.47 ± 4.24	71.18 ± 1.25	46.22 ± 3.69	70.10 ± 1.37	47.23 ± 3.76	70.54 ± 0.77	44.96[ab] ± 2.09	61.88 ± 2.46	54.91 ± 2.69	63.04 ± 2.46	53.35 ± 2.52	60.17 ± 2.36	51.73 ± 3.97	61.71 ± 1.37	53.33 ± 1.67
Overall mean	70.81 ± 0.73	44.22 ± 2.07	71.87 ± 0.72	48.50 ± 1.99	71.02 ± 0.79	46.02 ± 2.07			63.95 ± 1.47	55.78 ± 1.31	63.90 ± 1.34	54.79 ± 1.48	62.43 ± 1.31	53.78 ± 1.74		

Means bearing different superscripts differ significantly (P<0.05).

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Effect of KIEV, GPSE-1, and BL-1 Extenders On The Semen Quality Of Crossbred Boar*

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The quality and preservability of semen of crossbred boar having indigenous blood do not follow the same patterns as noticed in exotic breed. The present investigation was undertaken to study the preservability of crossbred boar semen in different extenders.

A total of 48 semen ejaculates, 12 from each of the 4 crossbred (Hampshire x local) boars aged 9 to 12 months were used in the study. Semen samples having more than 75 per cent progressively motile spermatozoa were diluted (1:3) with KIEV (Johnson *et al.*, 1981), GPSE-1 (Tamuli, 1982) and BL-1 (Johnson *et al.*, 1981) extenders following split sample technique. The KIEV extender was composed of glucose - 6 gms, sodium citrate dihydrate - 0.375 gm, Sodium bicarbonate - 0.120 gm, Sodium EDTA 0.375 gm and distilled water 100 ml. In GPSE-1 extender glucose-3.5 gm, Potassium sodium tartrate - 1.00 gm, Sodium citrate dehydrate-0.3 gm, Sodium EDTA-0.2gm and distilled water 100 ml. were incorporated. BL_1 extender consisted of glucose-2.97 gm, sodium citrate-1.00 gm, Sodium bicarbonate-0.20 gm, Potassium Chloride-0.03

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gm and distilled water 100 ml. The diluted semen was then preserved in 15 ml culture tube at 15°C in a BOD incubator. The preserved semen was evaluated for sperm motility, live sperm count and acrosomal abnormalities at 0, 24 and 48 hours of preservation.A drop of semen mixed with Kreb's ringer phosphate buffer (Grotjan *et al.*, 1975) was taken on a prewarmed slide. Little air was blown over the prepartion with an air blower and the sperm motility was assessed as per Zemjanis (1970). The percentage of live spermatozoa was estimated in a eosin-nigrosin stained smear as described by Beatty (1957) and modified by Tamuli (1982). The morphological abnormalities of acrosome were studied in stained smear using Giemsa stain as per Watson (1975). The data were analysed as per Snedecor and Cochran (1967).

The percentage of progressively motile sperm, live sperm and intact acrosome recorded in KIEV, GPSE-1 and BL-1 extenders at 0, 24 and 48 hours of preservation are presented in Table 1.

The variation in mean percentages of progressively motile sperm, live sperm and intact acrosome was highly significant between extenders, between preservation periods and due to interaction of extenders and preservation periods. Motility, live sperm and intact acrosome dropped significantly with the increase in duration of preservation. There was no significant difference in mean percentages of progressively motile sperm, live sperm and intact acrosome at 0 hour of preservation among the extenders but progressively motile sperm, live sperm and intact acrosome dropped significantly in BL-1 extender at 24 and 48 hrs of preservation than in KIEV and GPSE-1 extenders. Critical difference test revealed that there was no significant difference in progressive motility, live sperm and intact acrosome between KIEV and GPSE-1 extenders.

These findings are in agreement with those of Slaweta *et al.* (1981) and Tamuli (1982). Higher percentage of progressively motile spermatozoa, live sperm count and intact acrosome recorded in KIEV and GPSE-1 extenders might be due to difference in composition of extenders. All the three extenders used in the study showed more than 50% motile sperm upto 48 hrs. of preservation but KIEV extender maintained better sperm motility, live sperm count and intact acrosome.

Table 1 : Motility, live sperm count and intact acrosome (Mean*± SE) of crossbred boar semen at 0, 24 and 48 hours of preservation at 15°C in KIEV, GPSE-I and BL-1 extender.

Extender	Motility			Live sperm			Intact acrosome		
	0 hrs	24 hrs	48 hrs	0 hrs	24 hrs	48 hrs	0 hrs	24 hrs	48 hrs
KIEV	81.16^{a} ± 0.54	71.79^{b} ± 0.63	66.36^{c} ± 0.73	88.27^{a} ± 0.49	81.95^{bc} ± 0.45	76.13^{d} ± 0.49	90.92^{a} ± 0.39	83.50^{b} ± 0.43	77.83^{d} ± 0.64
GPSE-1	80.86^{a} ± 0.42	71.37^{b} ± 0.54	65.79^{c} ± 0.49	88.83^{a} ± 0.44	82.08^{b} ± 0.44	75.38^{de} ± 0.44	91.15^{a} ± 0.34	83.27^{bc} ± 0.48	76.92^{d} ± 0.59
BL-1	80.79^{a} ± 0.44	71.29^{b} ± 0.69	62.75^{d} ± 1.22	87.60^{a} ± 0.44	80.58^{c} ± 0.14	73.00^{e} ± 0.83	91.21^{a} ± 0.39	81.94^{c} ± 0.53	74.33^{e} ± 0.73

* Means bearing different superscripts for each parameter differ significantly.

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Ovulatory Disturbances In Bovines

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ABSTRACT

A total of 85 repeat breeder buffaloes and cows including heifers were investigated for ovulatory disturbances. An incidence of 17.94, 9.04, 14.28, 0.0% delayed ovulation and 12.82, 36.36, 10.71, 42.85 % anovulation was recorded in buffaloes, buffalo heifers, cows and cow heifers respectively, on the basis of clinical examination at different intervals. The ovulatory disturbances were more (34%) in buffalo group than cattle group (28.57%), with an overall incidence of 31.76% in repeat breeder bovines.

* * *

Ovulatory disturbances - delayed ovulation and anovulation are important aspects of infertility leading to failure of conception and repeat breeding leading to prolonged intercalving period, affecting the total production of milk and calf crop. Van Rensburg (1956) investigated the role of delayed ovulation and anovulatory estrus in the etiology of functional infertility. In his study of 163 cows, ovulation was delayed in 30 and failed completely in 17 animals. An incidence of 18% delayed ovulation has been reported in South Africa (Van Rosenburg and Devose, 1962). Choudhary *et al.* (1965) reported higher failure of ovulation in heifers (56.88%). Raizada (1981) observed 30% anovulatory estrous in Murrah buffaloes. Kavani (1984) reported 16.56% delayed and 15.09% anovulatory conditions in repeat breeding buffaloes.

Materials and Methods

A total of 85 repeat breeder buffaloes and cattle including heifers were clinically investigated at the Institute A.I. centre to study the ovulatory disturbances. Careful rectal

examination was carried out for location of "Graafian follicle" and "Regressing C.L." on the ovaries, alongwith degree of uterine tone. After examination and confirming estrus, the animal was inseminated. The second Gynaecological examination was carried out after 24 hours on the same lines. In case the animal was found again in estrus and if ovulations had not occurred the animal was reinseminated. Third examination was carried out after 48 hours, and if still the follicle was present, it was diagnosed as a case of "delayed ovulation". The same animal was called for examination between 8th to 10th day post-estrus for confirmation of ovulation and development of corpus luteum. If corpus luteum was not found on the ovary in which graafian follicle was present the case was diagnosed as "Anovulatory estrus".

Results and Discussion

The incidence of delayed ovulation in buffaloes, buffalo heifers, cows and cow-heifers was 17.94, 9.09, 14.28 and 0.0 percent respectively, while the corresponding figures for anovulations were 12.82, 36.36, 10.71 and 42.85% (Table-1). The overall incidence of delayed ovulation in buffaloes and cows was found to be 16 and 11.43% respectively, while the corresponding incidence for anovulation was 18% and 17.14% (Table-2).

In bovines the overall incidence of delayed ovulation and anovulation was 14.11 and 17.65% respectively. The occurrence of delayed ovulation and anovulation was more in buffaloes than cows. The results further revealed that anovulation was more prevalent in heifers than cows and buffaloes. The overall ovulatory disturbances in buffaloes and cows were 34 and 28.57% respectively with an average of 31.76% in bovines (Table-2).

In ovulatory disturbances rupture of the graafian follicle and liberation of the ovum either does not occur at all or is delayed with failure to conceive, due to non-survival or loss of fertilizing capacity of the sperm in the female genitalia. The essential feature of this syndrome is failure of the follicle to discharge the ovum within the recognised post-estrus period. This primary aberration may then give rise to either (i) anovulation where the follicle may either regress or develop into a follicular cyst, or (ii) delayed ovulation where there is ovulation at an abnormally long period after the termination of estrus.

It is presumed that in delayed ovulation the level of LH secreted by the anterior pituitary is just below the requirements for normal ovulation. In anovulation, there is still greater deficiency of LH so that ovulation is completely inhibited and regression takes place. Between these two, there sometimes appears to be an intermediate stage in which LH is insufficient to cause ovulation but nevertheless adequate to produce luteinisation of the follicle.

Acknowledgements

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Table 1 : **Incidence of ovulatory disturbances in repeat breeder bovines**

Species	Total No.of observations	Delayed ovulation		Anovulation	
		No.of observations	Percentage	No.of obervations	Percentage
Buffaloes	39	7	17.94	5	12.82
Buffalo heifers	11	1	9.09	4	36.36
Cows	28	4	14.28	3	10.71
Cow heifers	7	–	–	3	42.85

Table 2 : **Overall incidence of ovulatory disturbances in bovines.**

Species	Total No.of observations	Delayed ovulation		Anovulation		Total ovulatory disturbances	
		No.of observations	Percentage	No.of observations	Percentage	No.of observations	Percentage
Buffaloes	50	8	16.00	9	18.00	17	34.00
Cows	35	4	11.43	6	17.14	10	23.57
Total (in bovines)	85	12	14.11	15	17.65	27	31.76

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Cortisol Level in Normal and Abnormal Gestation in Cattle

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ABSTRACT

The level of cortisol in the cows with normal gestation and prolonged gestation was estimated to find out the possible relationship of this hormone in the process of parturition. The level of cortisol before and after parturition in normal pregnancy was significantly higher ($P<0.01$) as compared to abnormal parturition. The level of cortisol continued to be higher even after parturition when the sample was taken within 24 hours.

* * *

Corticosteroids, both in foetal and maternal circulation play an important role in inducing parturition. More specifically, the foetal and maternal corticoid levels increase very rapidly towards the last phase of pregnancy and reaches peak towards the preparturient period (Adams and Wagner,1970 and Schmitt *et al.*, 1975).The onset of parturition was characterised by a dramatic rise in blood corticoid levels in the cow and there is sudden fall in the level immediately after parturition (Hudson *et al.*, 1976; Hunter *at al.*, 1977 and Prakash and Madan,1985). Some alteration in the plasma cortisol level is thought to occur in cows having abnormal pregnancy resulting in prolongation of the gestation period. The present investigation was

therefore carried out to find out the possible relationship of plasma cortisol level in abnormal gestation of cattle.

Materials and Methods

Clinical cases of abnormal gestation having prolongation of gestation period beyond 280+30 days, were taken for the present study. A total number of 20 such cases presented in the Central Clinics of Orissa Veterinary College and in the Livestock breeding farm, Khapuria, were selected and investigated. Cows and heifers having normal gestation period (15 cows) were taken at random as control.

Blood was collected on the day of clinical investigation from cattle with abnormal gestation and one day prior to the expected date of parturition in control.Blood (15ml) was transfered to heparinised vial immediately and stored on ice until plasma was harvested by centrifugation. The heparinised sample was centrifuged at 1500 RPM at 4°C for 10 minutes in the refrigerated centrifuge and the plasma was collected. Another sample of blood was collected within 24 hours of expulsion of foetus in clinical cases after treatment, and within 24 hours of normal parturition in control cases.

Parturition was induced by injecting 24mg of Dexamethasone (Curadex injection/Concept Pharmaceuticals Pvt. Ltd./Composition: Dexamethasone (2ml) containing 4mg/ml) followed by 70mg of Stilboesterol Dipropionate, Vetostrol injection M & B Ltd.Composition: Stilboesterol Dipropionate (10ml) containing 10 mg/ml, by I.M. route. Plasma cortisol was estimated in the samples of abnormal gestation before and after parturition and also in control cattle by Fluorimetric method (Mattingly, 1962).

Results and Discussion

The level of plasma cortisol (mcg/dl) before and after parturition in normal pregnancy (control) and before and after induced parturition in prolonged gestation cases (clinical cases)is presented in Table 1.

Table 1: **Level of Plasma Cortisol (mcg/dl) in normal and prolonged gestation cows (Mean ± S.E.)**

Condition	Before parturition	After Parturition
Normal Gestation (Control) N = 15	19.200 ± 0.613	23.100 ± 0.754
Abnormal Gestation (Clinical cases) N = 20	19.330 ± 0.436	4.868 ± 0.497

N = No. of animals

Table 2 : **Test of Significance (Fisher 't' test) of plasma Cortisol levels in normal and abnormal gestation cases.**

Source	' t ' values
NBP X NAP	3.81**
NBP X PGBP	11.53**
NAP X PGAP	20.18**
PGBP X PGAP	8.08**

** P<0.01 significant at 1% level

NBP - Normal before parturition

NAP - Normal after parturition

NAP - Prolonged gestation before parturition

PGAP - Prolonged gestation after parturition

The test of significance ('t' test) was done to find out the difference between the levels in both conditions and the values are presented in Table 2.

In the present study the level of plasma cortisol was significantly (P<0.01) higher in normal parturition than that of prolonged gestation cases. There is substantial support to

these findings as evidenced from the field studies of naturally occurring prolonged gestation cases emphasizing the vital role of foetal pituitary and adrenal cortex for initiation of parturition in female animals. During the last stage of parturrition there is an increased secretion of A.C.T.H. from the foetal pituitary which stimulates rapid growth of foetal adrenals leading to rise in the level of cortisols which ultimately enter into the maternal blood induces parturition by activating the production of PGF_2 alpha and coordinating endocrine profile of the female animals (Adams and Wagner, 1970; Arthur, 1975 and Kamimura *et al.* 1978).

The level of plasma cortisol in both normal and abnormal gestation showed a high significant difference ($P<0.01$) before and after parturition (Table 2). This is in close agreement with the findings of Hunter *et al.*, (1977) and Prakash and Madan (1985). Therefore, it is highly probable that the increased level of cortisol before term initiates normal parturition. But perusal of literature indicates cows with prolonged gestation usually carry abnormal foetuses with malformed pituitary and adrenal cortex. This pathological aberration of endocrine glands might deter the normal production of cortisol during the last stage of parturition resulting in cessation of usual labour in due time. It has also been observed during the present investigation that the foetuses obtained after the induction of parturition had none or less development of head, resulting in less stimulation of the foetal adrenal cortex.

Induction of parturition in prolonged gestation cases have been tried with success by parenteral administration of dexamethasone alongwith stilboesterol (Rodriguez, 1979 and Madhavan and Kurien, 1985). The use of exogenous estrogen might have certain priming effect in triggering the normal hormonal profile for initiation of parturition. It can therefore be suggested that in cases of prolonged gestation exogenous administration of dexamethasone and stilboesterol will stimulate the production of prostaglandin in intact endometrium causing luteolysis with subsequent parturition.

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Incidence Of Abnormal Termination Of Pregnancy In Jersey Cows

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Abnormal termination of pregnancy due to non-specific causes is an important parameter in assessing the adaptability of cattle particularly of exotic origin to the local climate. The present study was therefore undertaken in a purebred Jersey herd in Andhra Pradesh.

Materials and Methods

From the data on 769 calvings over a period of eight years (1977-84), the incidence of abnormal termination of pregnancy due to abortion (non-contagious), still-birth, dystokia and mummified foetus was estimated. The herd was negative for Brucellosis, and other specific diseases.

The climate was hot during summer (104°F) and moderate (87.1 to 88.7°F) during rainy and winter seasons.

The effects of parity (1 to 7) and seasons of the year (March to June - Summer; July to October - Rainy; November to February - Winter) on the incidence of various conditions was tested by Chi-Square analysis.

Results and Discussion

The overall incidence of abnormal termination of pregnancy among purebred Jersey cows was 14.17% comprising of abortions - 7.73%; still births - 5.72%; dystokia - 0.39% and mummified foetus 0.13%.

The incidence of abortion (non-specific) in this study was lower than 11.9% reported for Jersey Cows and higher than in Ongole - 3.0% and Sindhi - 3.3% (Narasimha Rao, 1982); Gir - 2.52% (Shukla *et al*, 1977), while it rose in crossbreds proportionately with increase in exotic inheritance: Ayrshire X Hariana - 3.3 to 7.6%, Ayrshire X Sindhi 7.3 to 13.4% (Sen *et al*, 1953), Jersey X Deshi 1.58 to 9.63% (Ramamohana Rao *et al*, 1976). This trend seems to reflect differences in genotype, environment and their interactions that are responsible for non-contagious abortions in different breeds of cattle.

A seasonal trend was also seen with higher frequency ($P<0.01$) of abortions particularly at 91 to 150 day stage during summer, while late stage abortions tended to be higher during rainy season. Seasonal effects on abortion were also observed in Indian cattle. Obviously the hot summer climate was the incriminating factor.

Still Births : The incidence of still births in the present herd was comparable to that of 5.7% (Narasimha Rao, 1989) and 4.9% (Arnold and Becker, 1956) in Jersey under Indian and North American conditions, respectively but higher than 1.3% in Ongole and 1.2% in Hallikar (Narasimha Rao, 1982), 1.1% in Sahiwal (Kaikini *et al*, 1977) illustrating that still births are more common in exotic breeds maintained under Indian conditions.

Still births were preponderantly high ($P<0.01$) among first and second calvers accounting for 52 and 18% of the total cases respectively. Highest and lowest incidence ($P<0.01$) was observed during rainy and summer seasons respectively. The reasons for this variation are obscure.

Dystokia : Dystokia was less compared to

5.03% in Jersey X Gir (Kaikini *et al*, 1977). The relatively low birth weights may be attributed for the lower incidence of dystokia in Jersey Cows.

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A Note On Bacteriological Studies Of Cervical Secretion Of Infertile Cows And Buffaloes.

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Uterine infection in cows and buffaloes is one of the vital factors which leads to heavy economic losses in dairy industry. Various preparations of antibiotics and sulpha drugs are in use for the treatment of uterine infection. However, indiscriminate use of antibiotics invariably results in the development of resistant bacterial strains complicating the effectiveness of therapeutic measures.

The present investigation was carried out to study the microbiological incidence of uterine infection in infertile cows and buffaloes and sensitivity pattern of isolates against commonly used chemotherapeutic agents.

Cervical secretion was collected aseptically in sterilized sheath (Dabas and Maurya, 1988) from infertile cows (70) and buffaloes (53) brought to Veterinary Clinics of this University. Samples were subjected to bacteriological examination using blood agar plates in order to obtain isolated colonies of bacteria. After incubation for 24 hr at 37°C, colonies of Gram positive staphylococci were marked and fished out by platinum wire loop and subcultured in nutrient broth and reincubated for 24 hr. Gram negative colonies that appeared on blood agar plates were picked up and streaked on MacConkey agar plates. These were purified and identified according to Buchanan and Gibbons (1974).

Sensitivity/resistance pattern of isolates against 12 antibacterial agents was studied according to Cruickshank (1968). The chemotherapeutic agents (conc/disc) included Ampicillin (10 mcg), Oxytetracycline (30 mcg), Tetracycline (30 mcg), Erythromycin (15 mcg), Streptomycin (10 mcg), Neomycin (30 mcg), Penicillin (10 unit), Nitrofurantoin (30 mcg), Gentamycin (10 mcg) and Co-trimexazole (25 mcg).

20 animals (11 cows and 9 buffaloes) were

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kept as control, in which no exmination was conducted on cervical mucus. Intrauterine infusion of Oxytetracycline was given for 6 days in this group.

Out of 123 cervical samples, a total of 88 (71.5%) samples - 46(52.2%) from cows and 42(47.7%) from buffaloes were positive to bacterial infection. Gram positive cocci (staphylococci), Gram negative rod and mixed infections were isolated from 64.4%, 6.7% and 28.9% cases, respectively.

The majority of mucous samples were found sensitive to Chloramphenicol (93.2%), Ampicilline (90.9%), Nitrofuratoin (90.5%) and Erythromycin (88.6%). Less than 10% samples showed variable degree of sensitivity against penicillin, co-trimexazole, oxytetracycline and tetracycline.

The intra-uterine infusion alongwith parenteral injection of the most effective drug(s), either single or in combination of two, for 6 days at the time of estrus, resulted in conception in 67 (84.8%) animals within next two estrous cycles, when animals were inseminated with liquid semen. No animal in the control group recovered during this period. Thus from the present study it is concluded that microbiological examination and antibiotic sensitivity testing of infertile cows and buffaloes should be carried out in order to overcome genital tract infection.

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Seasonal Variations In The Thyroidal And Adrenal Cortical Hormones In Prepubertal And Pubertal Buffalo-Heifers

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ABSTRACT

The investigations were aimed at studying hormonal variations during different seasons in prepubertal and pubertal buffalo-heifers. Hormones were estimated by RIA technique. The plasma thyroxine level was maximum (68.96 ± 3.45 ng/ml) during winter season and minimum (48.36 ± 1.44 ng/ml) during autumn season in pubertal buffalo-heifers. The plasma triiodothyronine of buffalo-heifers. Thyroidal hormones showed a significant ($P<0.01$) variation among seasons. The plasma cortisol level was found to be significantly ($P<0.05$) high during

summer season in prepubertal buffalo-heifers. Overall climatic changes affected the thyroidal and cortisol hormonal levels.

* * *

The productive and reproductive performance of animals are markedly affected by the changes in macro and micro environments, which alter the hypthalmo-hypophyseal axis, thus affecting the thyroid and adreno-cortical functions. Under tropical conditions study on the role of thyroid and adrenal glands in relation to reproduction in buffaloes is of great importance for augmenting their reproduction. The thyroid and adrenal glands play very important role in various body functions, which in turn make the animals adjust to the adverse environmental conditions. The information on circulating level of thyroxine, tyriiodothyronine and cortisol hormones in relation to seasonal variations in buffaloes is lacking in literature, thus this study was undertaken in buffalo-heifers.

Materials and Methods

The study was conducted on 12 apparently healthy Murrah prepubertal (20-25 mths) and pubertal (26-30 mths) buffalo-heifers maintained under standard managemental conditions at PAU dairy farm. The study was undertaken during autumn (August to October -Av. temp. 34.6°C), winter (November to January -Av. temp. 22.8°C), spring (February to April, -Av. temp. 28.5°C) and summer (May to July, -Av. temp. 37.2°C) seasons. The maximum and minimum relative humidity (RH) observed during the experimental period was 94, 44; 90, 46; 77, 41 and 74, 42 percent in autumn, winter, spring and summer seasons respec -tively. Blood samples were collected at fortnightly intervals from jugular vein under aseptic conditions in heparinized vials. Plasma was separated by centrifugation at 2000 g for 30 minutes and stored at -20°C in different aliquots for further analysis. The plasma thyroxine (T_4) was estimated by RIA technique of Abraham (1977) using polyethylene glycol as precipitating reagent. The plasma triiodothyronine (T_3) was measured as per Chopra *et al.* (1971), using single antibody technique and dextran coated charcoal as precipitating reagent for unbound fraction. T_3 and T_4. RIA kits were procured from BARC, Bombay. The plasma cortisol was quantified as described by Moore et al. (1985), using DPC, (Diagnostic Products Corporation, Los Angels) I^{125} RIA kits. The statistical analysis of data was carried out as per Snedecor and Cochran (1968).

Results and Discussion

Seasonal variations in the concentration of T_3, T_4 and cortisol hormones in buffalo-heifers has been depicted in Table 1.

The maximum and minimum plasma thyroxine level was 68.96 ± 3.45 and 48.36 ± 1.44 ng/ml during winter and autumn season respectively in pubertal buffalo-heifers, whereas it was maximum during spring season in prepubertal buffalo-heifers, which is allied to the findings of Refsal *et al.* (1984) and Johnson and Yousef (1966) in cows. A significant ($P<0.01$) variation in thyroxine level was observed between different age groups and season as well. Thyroxine concentration was higher during winter season as the low temperature results in increase in thyrotropic activity. These observations are similar to the findings of Kapoor *et al* (1974). The plasma triiodothyronine (T_3) concentra- tion in prepubertal and pubertal buffalo-heifers was significantly ($P<0.05$) higher during winter was compared to other seasons (Table 1). Refsal *et al.* (1984) also reported significantly higher levels of T_3 in winter months in all cattle age groups. Pathak *et al* (1989) have also reported higher thyroid activity in female calves than male calves, possibly due to

greater body surface area and higher basal metabolic rate. The low concentration of T_3 during spring and autumn seasons is indicative of negligible stress on thyroid functioning during these seasons as thyroid functioning is dependent on environmental temperature. The thyroidal hormone concentration was low during autumn season, possibly due to high humidity.

The relationship between thyroidal functions and hypothalmo-hypophyseal-thyroidal axis in buffaloes is not fully elucidated. As the thyroidal hormones have a role in the maintenance of basal metabolic rate (BMR) and adjust to the body environmental conditions, ovarian-cyclicity and various reproductive processes warrant further investigation.

The plasma cortisol level was significantly ($P<0.05$) high (2.21 ± 0.63 ng/ml) during summer season as compared to other seasons in prepubertal buffalo-heifers which is quite in tune with findings of Borgohain *et al.* (1986), whereas in pubertal buffalo-heifers the cortisol concentration varied non-significantly. The plasma cortisol concentration was significantly ($P<0.01$) different between the two age groups of heifers. Thompson *et al.* (1963) also reported an increase in adrenal cortical hormones from 1.7 ug/dl to 4.5 ug/dl during controlled hot conditions as compared to cool conditions. The secretion of cortisol is controlled almost entirely by ACTH hormone secreted by adenohypophysis and any type of physical or thermal stress leads to enhanced ACTH secretion and consequently cortisol as well (Guyton, 1981). Hence high environmental temperature during summer season could be one of the reasons for higher cortisol concentration in buffalo-heifers.

Table 1 : **Average concentration (ng/ml) of plasma thyroxine, triiodothyronine, and cortisol during different seasons in buffalo-heifers.**

GROUPS	SEASONS			
	AUTUMN	WINTER	SPRING	SUMMER
		THYROXINE		
Prepubertal-heifers	47.43^{a} ± 2.48	61.60^{b} ± 2.79	64.18^{b} ± 2.59	61.55^{b} ± 2.15
Pubertal-heifers	48.36^{a} ± 1.44	68.96^{b} ± 3.45	63.20^{bc} ± 3.36	54.43^{ac} ± 2.34
		TRIIODOTHYRONINE		
Prepubertal-heifers	1.14^{a} ± 0.15	2.96^{b} ± 0.15	1.55^{a} ± 0.21	1.25^{a} ± 0.06
Pubertal-heifers	1.18^{a} ± 0.15	2.85^{b} ± 0.26	0.88^{ac} ± 0.07	1.11^{ad} ± 0.05
		CORTISOL		
Prepubertal-heifers	0.61^{a} ± 0.08	0.79^{a} ± 0.08	0.67^{a} ± 0.07	2.21^{b} ± 0.63
Pubertal-heifers	0.87a ± 0.12	1.15^{a} ± 0.09	1.41^{a} ± 0.25	1.23^{a} ± 0.32

Values with different superscripts differ significantly ($P<0.05$) among the groups.

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A Comparative Study Of Minerals In Buffalo (Bubalus bubalis) Cervical Mucus And Blood Serum vis-a-vis Progesterone

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ABSTRACT

Samples of blood and cervical mucus from Murrah buffalo heifers and buffaloes, 6 each were analysed for various minerals and progesterone (PG) levels during summer months.

Sodium was found to be significantly different in buffalo heifers and buffaloes blood serum as well as their cervical mucus, whereas K^+, Ca^{++}, Fe^{++} and Zn^{++} were found to differ only between blood serum and cervical mucus. Na^+, Mg^{++}, Fe^{++}, Cu^{++} and Zn^{++} were positively correlated between cervical mucus and blood serum, while K^+ and Ca^{++} were negatively correlated. Except Na^+ and Zn^{++}, all minerals were positively correlated to circulating PG. However, only Mg^{++} in the

cervical mucus was found to be positively correlated and all other minerals were found to be negatively correlated. These findings lend support to the concept that the cervical mucus is the product of active secretions.

* * *

The importance of cervico-vaginal and uterine fluids in the female reproductive tract is well recognised. The minerals play a significant role in the normal functions of domestic animals. Deficiency and excess minerals adversely affect reproduction (Hidiroglou, 1979). Ovarian hormones affect the properties of cervical mucus (Agarwal and Datta, 1976). Scanty information is available on the concentrations of minerals in cervical mucus and blood in relation to hormones. The present study is on minerals in cervical mucus and blood serum in relation to circulating PG levels.

Materials and Methods

Experimental : 12 cycling Murrah buffalo heifers and cows (six each) maintained under normal feeding and managemental conditions at PAU Dairy Farm, Ludhiana, were used. All were gynaeco-clinically normal at the time of sampling.

Blood and cervical mucus samples were collected on the day of estrus during summer months of April through July when the mean ambient maximum, minimum temperature (°C), relative humidity (%) and wind velocity (Km/hr) were 35.1, 22.7, 57 and 6.4, respectively. All the collections were made in the morning (6 to 8 A.M.). Blood was collected by jugular vein puncture, allowed to clot and serum was separated at 4°C by centrifugation at 3000 x g for 15 minutes. Serum thus obtained was stored in aliquots at -20°C till further analysis. Similarly, samples of cervical mucus were also kept.

Analytical : Sodium and potassium in both blood serum and cervical mucus were determined flame photometrically, while calcium, magnesium, iron, copper and zinc were estimated on atomic absorption spectrophotometer. The serum PG was measured by RIA technique (Ahmed et al. 1977). Progesterone anti-serum was a gift from James Hixon and Willium Hansell Cornell University, Ithaca, New York (USA) and the sensitivity per tube was 100 pg. The data were subjected to analysis of variance and correlations worked out (Snedecor and Cochran, 1967).

Results and Discussion

The mean values of PG and various mineral elements are presented in Table 1. The progesterone levels in the blood serum of buffalo heifers and cow on day of estrous were found to be 0.395 ± 0.07 and 0.302 ± 0.85 ng/ml, respectively. These values are in close agreement to those reported by Singh *et al.* (1979).

The sodium content in the blood serum of buffalo heifers and cows was 182.83 ± 1.17 and 184.33 ± 2.292 mcg/L, respectively. The concentration was within the normal range in buffaloes. However, Dabas *et al.* (1987) reported little lower values. Shultz *et al.* (1971) also reported lower values in cattle, ranging between 135.83 and 193.50 mcg/L in the cervical mucus, lowest being in the heifers.

The differences were found to be highly significant ($P<0.05$). The values are in agreement to those reported by Agarwal and Datta (1979). Similar results have been reported in cattle (Shultz *et al*, 1971; Goel *et al*, 1974). In heifers, it also differed significantly ($P<0.05$) between cervical mucus and blood serum. Low content in heifer could be due to different hormonal status (Goel et al. 1964). Guay (1966) observed increased concentration of Na^+ concurrent with sharp

decline in K^+ at estrus and Guay and Lamethe (1969) recorded a highly significant difference in the concentration of Na^+ in cervical secretions of normal than that of repeat breeders.

The potassium content was found to be 7.4 and 6.53 mcg/L in the blood serum of buffalo heifers and cows, respectively. The respective values in the cervical mucus were 8.78 and 12.53 mcg/L. The differences between cervical mucus and blood serum were highly significant ($P<0.01$). Shultz *et al.* (1971) also observed similar differences between bovine uterine fluid and blood serum. The blood values were little higher than those of Dabas *et al.* (1987).

The values in cervical mucus were found to be lower than those of Agarwal and Datta (1979) but are in agreement to those reported for bovine cervical mucus (Goel *et al*, 1974). The K^+ levels are under the hormonal control. Increased K^+ in cervical mucus is conducive to sperm longevity as it slows O_2 uptake and metabolism thus in turn help in capacitation (Rawat, 1979).

Calcium concentration of the blood serum varied between 9.33 ± .21 and 9.63 ± 0.30 mg/dL, while in cervical mucus it ranged from 5.07 ± 0.49 to 7.85 ± 0.43 mg/dL. These values differed significantly ($P<0.05$) between blood serum and cervical mucus. Similar results have been reported by Shultz *et al.* (1971), Dabas *et al.* (1987) and is less influenced by various intake of minerals and remained within normal limits 9-12 mg/dL (Underwood, 1971).

Magnesium levels in both, cervical mucus and blood serum varied insignificantly from 3.08 ± 0.28 to 3.68 ± 0.25 mg/dL lowest being in cervical mucus and highest in blood serum of heifer. Magnesium content was found to be well within the normal range of 2–5 mg/100 ml. These values agree with those reported in CM (Goel *et al.* 1974) and Blood Serum (Dabas *et al.* 1987). However, Parshad *et al.* (1979) reported slightly lower values.

Although the physiological significance of trace elements in general and in reproduction is well-established (Hidiroglou, 1979), yet little is known about their physiological significance in the cervical mucus. The mean circulating level of iron varied from 0.035 ± 0.005 to 1.35 ± 0.20 mg/dL, lowest being in the serum of buffalo cow and highest in the cervical mucus of heifer. The differences between blood serum and cervical mucus were found to be highly significant ($P<0.01$). Copper content was high 0.150 mg/dL ($P<0.05$) in blood serum than cervical mucus (0.032 mg/dL). Likewise, Zinc levels were also high in the blood than cervical mucus and varied significantly from 0.120 ± 0.024 to 0.193 ± 0.017 mg/dL. It is interesting to note that the concentration of iron in the cervical mucus is significantly higher than that in the blood (Table 1) which again lend support to the concept that cervical mucus is not only the product of simple diffusion but of active secretions.

Iron level is affected by the physiological state of the animal (Parshad *et al*, 1979). High level of iron in the cervical mucus could be of some importance for sperm motility and survival in the female reproductive tract.

The average circulating level of copper was found to be similar to Vadnere and Singh (1989) and lower than reported by Parshad *et al.* (1979) and Dabas et al. (1987). Low level of serum copper has been associated with low fertility (Rowland et al, 1977). Copper and iron affect fertility (Desai *et al*, 1982). Serum copper reflect the hormonal activity in animals (Seto and Henkins, 1973). Likewise zinc level is known to affect ovarian activity (Sharma *et al*, 1988). Dufty *et al*, (1977) reported that plasma zinc concentration was little affected at various stages of estrous cycle. Krichgessner et

al. (1976) concluded that oestrous behaviour of cattle and level of gonadotropin hormones in the serum were affected with dietary Zn^{++} deficiency.

Sodium, magnesium, iron and zinc showed positive correlation (4, .35, .06, .30 and .44, respectively) while potassium, calcium and copper had negative correlations (r, .51, .22 and .15) between cervical mucus and blood serum. The PG content was negatively correlated (range, r, .1 to .44) with all the minerals except magnesium (r, .38) in the cervical mucus while it was postiviely correlated in blood serum, but for sodium (r, .21) and zinc (r, .29). It was significantly correlated (r, 0.68 $P<0.01$) with potassium in blood. From these studies, it could be inferred that the secretions in the cervical mucus are largely dependent upon hormonal activity.

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Table 1 : The Mean ± SEM Progesterone and some of the mineral elements in cervical mucus (CM) and Blood Serum (BS) at mid-estrous of buffalo heifers (H) and buffalo cows (C)

Element	Cervical mucus		Blood serum		Statistical significance between	
	buffalo heifer	buffalo cow	buffalo heifer	buffalo cow	H & C	BS & CM
Progesterone (ng/ml)	–	–	0.395 ± 0.07	0.302 ± 0.05	NS	–
Sodium (m Eq/L)	135.83 ± 9.20	193.50 ± 9.82	182.83 ± 1.17	184.33 ± 2.92	**	*
Potassium (m Eq/L)	8.78 ± 0.54	12.53 ± 1.06	7.4 ± 0.52	6.53 ± 0.46	NS	**
Calcium (mg/dl)	7.85 ± 0.43	5.07 ± 0.40	9.33 ± 0.21	9,63 ± 0.30	NS	*
Magnesium (mg/dl)	3.08 ± 0.28	3.48 ± 0.13	3.68 ± 0.25	3.30 ± 0.10	NS	NS
Copper (mg/dl)	0.032 ± 0.008	0.032 ± 0.005	0.152 ± 0.012	0.147 ± 0.01	NS	*
Iron (mg/dl)	1.35 ± 0.20	0.78 ± 0.09	0.62 ± 0.009	0.035 ± 0.005	NS	**
Zinc (mg/dl)	0.137 ± 0.031	0.120 ± 0.024	0.193 ± 0.017	0.170 ± 0.016	NS	*

Each figure represents the mean value for six animals done in duplicate
* $P<0.05$
** $P\leq0.01$

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Studies On Certain Laboratory Tests For Pregnancy Diagnosis In Buffaloes

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ABSTRACT

Several laboratory methods for diagnosing pregnancy were tried in buffaloes. Out of the tests carried out on cervical mucus, the over-all accuracy of 80.87, 77.27 and 64.00 % was recorded in specific gravity, sodium hydroxide and distilled water tests respectively. In copper sulphate test with milk samples, the accuracy was 60.24%, whereas with barium chloride urine test,it was 64.51%. It was concluded that these tests have a limited application in early diagnosis of pregnancy.

* * *

Pregnancy diagnosis provides the means of identifying and combating fertility problems. It assists in economy and efficiency of herd management by identifying non-pregnant animals. It also gives a clue to early embryonic death rate and more accurate information about conception rates. The present studies were undertaken to find out simple and reliable laboratory tests for diagnosing early pregnancy in buffaloes.

Material and Methods

Present studies were carried out on test materials - milk and urine of 284 Nagpuri and Murrah buffaloes, obtained from local abattoir, University Livestock Instructional Farm, Akola and Cattle Breeding Farm, Telankhedi, Nagpur. Cervical mucus was obtained only from abattoir specimens.

1. **Cervical Mucus:** In all 191 samples of mucus were subjected to one, two or all the three of the following tests, depending upon the quantity of mucus obtained.

(i) **Specific Gravity Test** : 183 samples of cervical mucus were subjected to this test. 10 ml. of copper sulphate solution of 1.008 specific gravity was taken in test tube and 0.5 ml. of cervical mucus was dropped in it. The test was considered positive for pregnancy if the mucus sank to the bottom. Results were compared with findings recorded on actual palpation of the genitalia after slaughter of each animal.

(ii) **Sodium Hydroxide (NaOH) Test** : 110 samples were subjected to this test. 0.5 ml. of cervical mucus was dissolved in 5 ml. of 10% NaOH solution in test tube and boiled for six minutes in hot water bath. Development of a clear light brownish colour was indicative of pregnancy and pale yellow colour for non-pregnancy.

(iii) **Distilled Water Test** : 25 samples were subjected to this test, wherein 0.5 ml. of cervical mucus was boiled in 5 ml. of distilled water for 3 minutes. If the mixture turned cloudy, the test was recorded positive for pregnancy and negative when it remained transparent.

2. **Milk- Copper Sulphate ($CuSO_4$) Test:** 249 milk samples from 83 buffaloes were subjected to $CuSO_4$ test at the rate of three tests per buffalo at weekly intervals. 0.5 to 1 ml. of milk was stirred in 10 ml. of 3% $CuSO_4$ solution. If the milk coagulated, the test was recorded as positive for pregnancy and negative when it remained homogenous for several hours. Results were compared with those of P.R. examination.

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3. Urine : Barium Chloride Test :

178 tests were carried out in 62 buffaloes. To 5 ml. of freshly collected and filtered urine, 4 to 5 drops of 1% Barium Chloride solution was added and the test tube allowed to stand for three minutes. If the urine samples remained clear for several hours, the test was considered positive and negative if it developed a white precipitate. Results of this test were also verified on P.R. examination.

Results and Discussion

Accuracy of cervical mucus specific gravity test in early pregnancy was 80.00% and for middle and advanced pregnancy it was 100% each. This indicates that, accuracy of the test increases with the advance in gestation period. Hence the test has a limited application for diagnosing early pregnancy which is in agreement with Babiceva (1966). Out of the total 171 non-pregnant buffaloes subjected to this test, 80.11% were diagnosed correctly. This finding is in agreement with that of Temblador and Canta (1971) although they reported slightly lower values. The overall accuracy of the test based on pregnant and non-pregnant animals was found to be 80.87% (Table 1) which is in agreement with Babiceva (1966).

Cervical mucus NaOH test gave 40% accuracy in early pregnancy, while 100% accuracy was obtained in cases of middle and advanced pregnancy indicating increasing its accuracy with advancement of pregnancy. This finding is in agreement with that of Hukeri and Singh (1968) and Sethumathavan and Raja (1971) in cows. Out of 99 non-pregnant buffaloes, 77 (77.77%) were diagnosed correctly by this test. However, this finding is not in agreement with Sethumathavan and Raja (1971) who reported 100% accuracy in non-pregnant cows. Overall accuracy of the test was found to be 77.27% (Table 1).

In cervical mucus - distilled water test, out of the three cases of early pregnancy, only one (33.33%) was diagnosed correctly. This shows wide difference with that of Berchtold and Bostedt (1970); Sethumathavan and Raja (1971) and Temblador and Calvan (1971), who reported higher values in cows. Both cases of middle pregnancy were diagnosed as non-pregnant, while 15 (75%) of the 20 non-pregnant were diagnosed correctly. The overall accuracy of distilled water test was found to be 64% which is lesser than that recorded by Tjupic and Kuznecov (1960) and Berchtold and Bostedt (1970) who reported 90 to 100% accuracy of this test. The difference might be due to the variation in the number of animals studied, species difference and methodology adopted.

Overall accuracy of milk $CuSO_4$ test was found to be 64.24% which is lower than the reports of Stancev and Angelov (1966) and Temblador and Acosta (1971) who reported 90.00 and 86.00% accuracy in cows. Although the test gave 60.24% accuracy quantitatively, it is insignificant qualitatively as all the 33 non-pregnant buffaloes were diagnosed to be pregnant.

Urine-Barium Chloride test revealed 68.96% accurancy in early and 100% in middle and advanced pregnancy cases.In nonpregnant animals,its accuracy was found to be 52% which is fairly in agreement with that of Temblador and Landa (1971).

Overall accuracy of the test was found to be 64.51% . This finding is not in agreement with that of Maslova and Smirnov (1965) who reported 95 to 100% accuracy.The difference between the present findings and those of previous workers may be due to low endocrine threshold in buffaloes as compared to cows.

Table 1 : **Efficacy of pregnancy diagnosis tests during various stages of gestation in buffaloes (percent)**

Sr. No.	Test	Stage of pregnancy			Non-pregnant	Over-all accuracy (%)
		Early	Middle	Advanced		
1	**Cervical Mucus**					
(i)	Specific gravity	80.00 (5)	100.00 (6)	100.00 (1)	80.11 (171)	80.87 (183)
(ii)	Sodium hydroxide	40.00 (5)	100.00 (5)	100.00 (1)	77.77 (99)	77.27 (110)
(iii)	Distilled water	33.33 (3)	00.00 (2)	—	75.00 (20)	64.00 (25)
2	**Milk**					
(iv)	Copper sulphate	100.00 (33)	100.00 (16)	100.00 (1)	00.00 (33)	64.24 (83)
3	**Urine**					
(v)	Barium chloride	68.96 (29)	100.00 (4)	100.00 (4)	52.00 (25)	64.51 (62)

Figures in the parenthesis indicate number of observations.

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Incidence Of Anoestrum In Murrah Grade Buffaloes (Bubalus bubalis) Of Calcutta Khatals.

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ABSTRACT

Of 1890 Murrah grade buffaloes in Calcutta city khatals, 961(50.84%) suffered from prolonged 'reported anoestrous' condition upto 240 days after last calving. 460 such buffaloes were further studied to ascertain the causes of prolonged anoestrous condition. The incidence of true anoestrum (no G.F or C.L.), sub-functional ovaries (standing G.F.), missed oestrus (functional C.L.), heat during examination (silent ovulation), pregnancy and persistent C.L. (PCL) was observed respectively in 154 (33.47%), 105 (22.82%), 166 (36.08%), 28 (6.08%), 4 (0.86%) and 3(0.65%) buffaloes.

* * *

Hundreds of pregnant and freshly calved high yielding buffaloes, particularly Murrah grade breed, are brought to Calcutta and suburbs from outside. At the end of lactation, they prove uneconomic due mainly to prolonged postpartum anoestrum and majority find their way to the slaughter houses. The present study was therefore undertaken to investigate the causes and incidence of prolonged postpartum anoestrum in these animals.

Materials and Methods

Studies were undertaken in 460 apparently normal buffaloes available in the private organised herds for investigating into the various causes of prolonged anestrus condition exceeding 240 days from last parturition. The studies were carried out in selected khatals by collecting information on their reproductive behaviour from the owners/ caretakers and also by detailed gynaeco-clinical examination once in every 7-10 days to assess their reproductive status.

Results and Discussions

Of the 460 healthy lactating buffaloes, 154 (33.5%) had smooth and inactive ovaries with no functional follicle or C.L. The uterus was relaxed and flaccid. There was no tonicity of uterus. The vaginal mucus membrane was dry, pale or anaemic and cervix closed with thick tenacious scanty mucus. The gynaecological findings indicated that 33.47% animals were sub-fertile with true anoestrum due to non-functioning ovaries. The finding nearly agrees with that of Luktuke *et al* (1978) who recorded 32.82% incidence in buffaloes. Kodagali (1968), Rao *et al* (1971) and Deshpande (1983) studied the incidence of anoestrus conditions in buffaloes under village conditions in different parts of the country and observed higher incidence of inactive or quiescent ovaries ranging from 41 to 45%. mainly due to lower level of nutrition in village herds as compared to the herds under study availing high nutritive production ration for high milk yield.

The overall incidence of 22.82% sub-functional ovaries with standing follicles was recorded. This is in close agreement with Luktuke et al (1973)and Luktuke et al (1978) who reported 23.15% and 22.09% incidence of sub-functional ovaries in abattoir and rural buffaloes causing anoestrum. Prolonged post-partum anoestrum due to sub-functional

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ovaries in the buffalo cows in high lactation may be due to insufficient ovulatory surge of L.H. which may result from inefficient synergistic action of progesterone and estrogen. In these high yielding buffalo cows, high level of prolactin concentration in the circulating blood suppressed the action of FSH and LH affecting growth and ovulation of the standing ovarian follicles (Heranjal *et al,* 1978).

The third major cause of missed heat was found to be highest (36.08%). The presence of palpable functional C.L. in the ovaries indicated that these buffaloes were undergoing internal cyclic changes but due to aberrations in estrus pattern and lack of external estrus symptoms, the owners or caretakers failed to detect heat. The considerable incidence of anoestrus resulting from unobserved or missed heat in large herds is common. Chauhan *et al,* (1979) observed 25.6% buffaloes in anoestrus condition due to unobserved heat. In the present study higher incidence of missed heat is due to maintenance of large herds of buffalo cows with no provision of teaser bull and the owners or caretakers solely dependent on the sexual behaviour of buffaloes for heat detection.

The studies further revealed that 28 (6.08%) buffaloes were detected in estrus during gynaeco-clinical examination. On palpation uterine comua were found turgid and hard. Cervical mucus was detected on passing the vaginal speculum. Kodagali (1968) observed a little higher incidence (8.76%) of silent ovulations in Jaffarabadi buffaloes under field conditions. 4 (0.86%) buffalo cows were found to be in early pregnancy.

In 3 (0.65%) buffalo cows large corpus luteum (CL) was palpated on the ovaries on repeated examinations, with uterus enlarged and soft. These animals were perhaps suffering from low grade infection which went undetected at the time of their selection and later developed into persistent corpus luteum (PCL).

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Seasonal Variations In Gonadal And Hypophyseal Hormones In Prepubertal And Pubertal Buffalo-heifers

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ABSTRACT

Study was aimed at investigating hormonal changes during different seasons in prepubertal and pubertal group of Murrah buffalo-heifers. Hormones were quantified by RIA technique. Plasma estradiol-17 B concentration was significantly high during winter season. Low estradiol-17 B levels were observed during summer and spring seasons. However, plasma progesterone levels were low during autumn and high during winter and summer seasons in both the groups. Circulating LH level was observed to be high: 9.32 ± 0.44 and 8.40 ± 0.30 m.i.u./ml in prepubertal and pubertal heifers respectively during winter as compared to other seasons.

* * *

The macro and micro-environments play a major role in the productive and reproductive performance of animals by affecting hypothalmo-hypophyseal-gonadal axis altering their endocrine profile which is the nucleus of almost all reproductive functions. The reproduc-tive physiology of buffalo has been poorly investigated, less so with respect to climatic variations. The present study was undertaken to monitor the seasonal variations in circulating levels of gondal and hypophyseal hormones in Murrah heifers.

Materials and Methods

Study was conducted on 12 apparently healthy Murrah prepubertal (20-25 mths) and pubertal (26-30 mths) buffalo heifers maintained under standard managemental conditions at the University Dairy Farm. Investigations were undertaken during autumn (August to October, Av. temp. 34.6°C); winter (November to January, Av. temp. 22.8°C); spring (February to April, Av. temp. 28.5°C) and summer (May to July, Av. temp. 37.2°C) seasons. The maximum and minimum relative humidity (RH) observed during the experimental period was 94, 44; 90, 46; 77, 41 and 74, 72% during autumn, winter, spring and summer seasons respectively. Blood samples were collected at fortnightly intervals from jugular vein under aseptic conditions in heparinized vials. Plasma was separated by centrifugation at 2000 g for 30 mts. and stored at -20°C in different aliquots for hormonal analysis. The plasma estradiol-17 B was quantified as per Abraham (1977) and plasma progesterone estimated as per Abraham (1981) , by solid phase radioimmunoassay (RIA) technique using RIA kits procured from DPC (Diagnostic products corporation, Los Angles, USA.). Plasma luteinizing hormone (LH) was assayed as per Peterson and Swendloff (1979) using RIA kits obtained from BARC (Bhabha Atomic Research Centre, Bombay). Intra-assay coefficients were found within the working range. Statistical analysis of data was carried out as described by Snedecor and Cochran (1968).

Results and Discussion

The concentration of estradiol-17 B was maximum (19.92 ± 2.61 pg/ml) during winter and minimum (6.86 ± 0.31 pg/ml) during autumn season in prepubertal and pubertal

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buffalo-heifers as compared to other seasons (Table 1). Analysis of variance also revealed a significant (P<0.01) seasonal variation in estradiol-17 B level in buffalo-heifers, which is quite similar to reported observations of Sane *et al* (1982). Average plasma progesterone level was significantly (P<0.05) high during summer as compared to autumn season in prepubertal buffalo-heifers. However, the progesterone (PG) concentration varied non-significantly in pubertal buffalo-heifers. Mills *et al.* (1972) also reported higher PG concentration during summer months in cows. Takkar *et al.* (1983) also observed significant seasonal variation in PG level in buffalo-heifers, which confirms our results.

The plasma LH concentration was significantly (P<0.05) high during winter and low during spring in both the groups of heifers. Maximum LH concentration was 9.32 ± 0.44 m.i.u./ml during winter season in prepubertal heifers. Analysis of variance revealed significant (P<0.01) seasonal variation in LH level though variation between groups was non-significant. Madan and Johnson (1973) while studying environmental heat effect on bovine LH level, observed a decline to the base level and peak of LH in plasma with rise in core temp. by 1 to 1.5°C in heifer. However, Janakiraman (1979) found a low LH during winter and high LH level in monsoon season in Surti buffaloes. This difference is possibly due to breed variations. Kaker *et al* (1980) reported slightly higher LH concentration during cooler months and lower during hotter months, which is similar to our findings.

Significant variations in the concentration of plasma estradiol-17 B, plasma PG and LH were observed during different seasons. Hence, it is postulated that hypothalmo-hypophyseal-gonadal axis plays a significant role in seasonal alterations of hormonal profile in buffalo-heifers.

Table 1 : Average plasma Estradiol-17 B, Progesterone and Luteinizing hormone concentration during different seasons in prepubertal and pubertal buffalo-heifers.

GROUPS	SEASONS			
	AUTUMN	WINTER	SPRING	SUMMER
		Estradiol-17 B (pg/ml)		
Prepubertal Heifers	6.86^{a} ± 0.31	19.92^{c} ± 2.61	11.94^{b} ± 1.37	8.66^{b} ± 0.57
Pubertal Heifers	8.01^{a} ± 0.97	17.04^{b} ± 1.54	15.77^{b} ± 1.65	9.38^{a} ± 0.63
		Progesterone (ng/ml)		
Prepubertal Heifers	0.49^{a} ± 0.16	1.33^{ab} ± 0.32	1.25^{ab} ± 0.27	1.31^{b} ± 0.23
Pubertal Heifers	0.69^{a} ± 0.19	1.33^{a} ± 0.36	1.30^{a} ± 0.22	1.33^{a} ± 0.21
		Luteinizing Hormone (m.i.u/ml)		
Prepubertal Heifers	7.17^{b} ± 0.15	9.32^{c} ± 0.44	6.06^{a} ± 0.38	7.96^{b} ± 0.13
Pubertal Heifers	6.87^{a} ± 0.11	8.40^{b} ± 0.30	5.43^{c} ± 0.53	7.74^{b} ± 0.17

Values with different superscripts differ significantly (P<0.05) within the group.

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Reproductive Disorders of Crossbred Cows Of Kerala

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ABSTRACT

3,427 crossbred cows were subjected to detailed gynaeco-clinical examination in the infertility camps (1981-86). The overall incidence of various conditions was: anoestrum (30.36%); suboestrum (19.13%); cystic ovarian degeneration (1.42%); vaginitis (3.68%); cervicitis (1.54%); endometritis (20%); salpingitis and bursal adhesions (1.07%); underdeveloped genitalia (12.92%); ovulatory disturbances (8.89%); ovarian hypoplasia (0.08%); extra-uterine pregnancy (0.04%); hydrometra (0.12%); mucometra (0.04%); free-martin (0.04%); mummified foetus (0.08%); uterine adhesions (0.04%) and metritis (0.08%). The remedial measures adopted and results obtained are also discussed.

* * *

Sexual or reproductive health control programme is an integral part of successful management of dairy cows. This is essential particularly when large scale dissemination of superior germplasm is being implemented for

accelerating the pace of dairy cattle production through A.I. To exploit the full potential of the superior progeny thus produced, it is imperative that strict scientific reprodutive programme is followed. Hence, periodic infertility camps are arranged in different parts of the State. The data has been classified and presented herein.

Material and Methods

The animals brought to the various infertility camps organised in different parts of the State during 1981-86 formed the material for the study. These animals were subjected to detailed gynaeco-clinical examination and the various conditions responsible for impaired fertility were studied and treated, the results of which were assessed in follow-up camps. For identifying the intensity of incidence of these conditions, the State was divided into three regions: south, central and north and data classified accordingly.

Results and Discussion

Out of the total 3,427 animals subjected to detailed investigation, 1.437 (41.93%) were from north, 1305 (38.07%) from central and 685 (19.98%) from southern part of Kerala. Perusal of the data revealed that 1042 animals out of 1437 (72.5%) in the northern region, 984 out of 1305 (75.40%) in central region and 504 out of 685 (73.57%) in the southern region were found infertile (Table 1). The rest of the animals were either pregnant, normally cycling or without any apparent abnormality.

The most important observation was the high incidence of anoestrum (30.36%), which was highest in the northern region (36.08%) and lowest in the central region (23.98%). Earlier reports also indicated anoestrum as the most common cause of infertility in crossbred cattle (Nambuthiripad, 1978; Mathew and Namboothiripad, 1979). Remedial measures taken by improving energy intake, administration of essential minerals and trace elements by providing feed supplements and administration of Gonadotrophic hormone releasing agents like clomiphene citrate in selected cases showed marked improvement as evinced by subsequent ovarian activity. The major cause of anoestrum was nutrition. Estimation of serum calcium, inorganic phosphorus, haemoglobin, copper, protein, glucose and calcium phosphorus ratio also confirmed this. Vast majority of these animals were weak, debilitated and exposed to qualitative and quantitative nutritional deficiency, besides deficiency of trace elements and minerals. This results in hormonal imbalance leading to anoestrum. Nutritional deficiency is the single major etiological factor for anoestrum in Indian cattle.

The incidence of underdeveloped genitalia and delayed maturity was 12.92% in heifers, possibly due to inadequate feeding and improper care. With improved feeding and better management practices, majority of the heifers resumed reproductive activity. Although detailed haematological studies could not be carried out in each case, based on the earlier reports of Pillai (1980), who observed low inorganic phosphorus (4 mg/dl), wide Ca:P ratio (2.69) and low copper (91.07 g/dl) in similar heifers, it is assumed that these animals also suffered from inadequate feeding. Most of these heifers were weak and debilitated.

Suboestrum was noticed in 19.13% animals. It was predominant (24.67%) in northern region and least (11.50%) in the south, since the animal husbandry activities are more active in the southern region, Earlier reports also reveal 23.07% suboestrum in Brown-Swiss crossbreds (Mathew and Namboothiripad, 1979) and 32% in Jersey crossbreds (Ghosh, 1982). A characteristic feature observed was that silent heat was more in aged animals and in debilitated and undernourished

heifers. Supplementation of minerals in the feed tended to correct the condition in most of the animals. Administration of PGF_2 alpha analogue in animals with suboestrum showed marked improvement with majority of animals exhibiting oestrum within 72 hours (Nair and Madhavan, 1984). Moreover, intensive measures for heat detection by educating farmers, reduced the incidence of suboestrum during later investigations.

Ovulatory distubances mainly delayed ovulation and few cases of anovulation were also noticed in 8.89% of animals. The complexity of climatic and other stress factors on the exotic crosses are yet to be understood fully. Higher incidence of ovulatory disturbances in crossbreds than indigenous cows could be due to adverse climatic and other stress factors (higher milk production). But these were observed only as temporary aberrations leading to spontaneous recovery in most cases. Rational treatment with LH always showed good results. Encouraging results were also noticed by administration of low dose (4 mg i/m) of progesterone in the beginning of oestrum as ovulation inducing agent. However, repeated inseminations in the same heat could effectively overcome the problem of delayed ovulation.

The overall incidence of cystic ovary (1.42%) was lower than previous reports of Mathew and Namboothiripad (1979). The higher incidence (2.85%) in central Kerala could be correlated to greater population of high yielding crossbed cows in that area. Iyer (1978) also reported higher incidence of cystic ovaries in high yielding crossbred cows. Higher incidence of cystic ovary due to stress factors can be justified in a large population of high yielding crossbred cows. LH therapy was found most successful in combating this conditon. However, the genetic nature of the condition cannot be overlooked.

Pathological conditions affecting the reproductive tract like vaginitis, cervicitis and endometritis constituted about 25% of total cases. Endometritis alone accounted for 20% of infertility. Infection appears to be the most common cause of infertility in cattle. Although specific infections can cause all the above conditions as reported earlier (Namboothiripad, 1978), non-specific infection also plays a major role in causing temporary infertility.

Post-partum complications without proper treatment leads to chronic endometritis with resultant reproductive failure of a permanent nature. Majority of cows exhibited resistance to common antibiotics as revealed by sensitivity tests. However, Gentamycin and Kanamycin were found effective in controlling most of the infections. This explains the danger of indiscriminate use of antibiotics without resorting to sensitivity test.

The incidence of permanent infertility due to congential or acquired conditions was low. Unilateral ovarian hypoplasia was observed in 0.08% animals whereas bilateral hypoplasia was nil. Incidence of salpingitis, ovario-bursal adhesions and uterine adhesions was negligible. About 5% of problem animals were infertile due to acquired, genetic or congenital causes.

Poor nutrition, defects in handling of semen, inadequacy of proper and timely A.I. and treatment will have to be reckoned as causes of infertility under the present circumstances. The essential prerequisite to ensure optimum fertility is better reproductive health control.

Table 1 : **Reproductive disorders in cross-bred cattle of Kerala State (1981-1986)**

Sr. No.	Item	Northern region	Central region	Southern region	Total
I.	Total No. of animals examined	1437	1305	685	3427
II.	No. of animals having reproductive disorders	1042	984	504	2530
III.	Reproductive disorders encountered				
1.	Anoestrum	376 (36.03%)	236 (23.98%)	156 (30.95%)	768 (30.36%)
2.	Suboestrum	257 (24.67%)	169 17.17%)	58 (11.50%)	484 (19.13%)
3.	Cystic ovarian degeneration	4 (0.38%)	28 (2.85%)	4 (0.79%)	36 (1.42%)
4.	Vaginitis	3 (0.28%)	24 (2.44%)	36 (7.14%)	93 (3.68%)
5.	Cervicitis	10 (0.96%)	15 (1.52%)	14 (2.78%)	39 (1.54%)
6.	Hydrometra	—	3 (0.30%)	—	3 (0.12%)
7.	Endometritis	154 (14.78%)	258 (26.22%)	94 (18.65%)	506 (20.00%)
8.	Salpingitis & bursal adhesion	3 (0.29%)	22 (2.20%)	2 (0.40%)	27 (1.07%)
9.	Underdeveloped genitalia & delayed maturity	114 (10.94%)	126 (12.8 %)	87 (17.26%)	327 (12.92%)
10.	Ovulatory disturbances	85 (8.16%)	94 (9.55%)	46 (9.13%)	225 (8.89%)
11.	Ovarian hypoplasia	—	2 (0.20%)	—	2 (0.08%)
12.	Extra uterine pregnancy	—	1 (0.10%)	—	1 (0.04%)
13.	Mucometra	—	1 (0.10%)	—	1 (0.04%)
14.	Freemartin	—	—	1 (0.20%)	1 (0.04%)
15.	Mummified foetus	1 (0.096%)	1 (0.1 %)	—	2 (0.08%)
16.	Uterine adhesion	—	—	1 (0.20%)	1 (0.04%)
17.	Metritis	—	2 (0.2 %)	—	2 (0.08%)
18.	Other Miscellaneous conditions	5 (0.48%)	2 (0.20%)	5 (0.99%)	12 (0.47%)

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Incidence And Treatment Of Various Forms Of Dystocia In Buffaloes

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Bovines are the most commonly affected species with dystokia, while its incidence in mare and sow is very low (Arthur *et al.*, 1982). Roberts (1986) reported various forms of foetal and maternal dystocia in cows. Incidence of dystocia in relation to season, age, breed, parity and sex of foetus has been recorded in cows (Verma and Mishra, 1984; Singla *et al.*, 1990). However, such reports are very scanty in buffaloes. So an attempt has been made to study the incidence and treatment of various types of dystocia in buffaloes.

A total of 77 buffaloes affected with dystocia (excluding uterine torsion) referred to the Vety. Clinics of this University, have been included in the present study. Incidence of various forms of dystocia and their relation with parity and sex of the foetus has been recorded. Depending upon the general conditions of the dam, form of dystocia and duration of its occurrence, efficacy of different modes of treatment was also studied.

Higher incidence of foetal dystocia (71.43%) as compared to maternal dystocia (28.57%) recorded in the present study, supports the observation of Tutt (1944) and Singla *et al* (1990) in cattle. Low incidence in first calvers as compared to pluriparous buffaloes is contradictary to the findings of Edward (1979) and Singla *et al* (1990). This may be ascribed to the fact that fewer and only complicated dystocias are referred to this clinics and simple cases are being treated in the field by the Veterinarians.

Male calves were found to be more frequently associated with difficult calvings as compared to females (Table 1). These findings corroborate with the observations of Edward (1979) and Singla *et al* (1990).

Narrow pelvis, monstrosities, insufficiently dilated cervix and complicated dystocia were the main conditions where caesarean section was performed and 72% success achieved. However, manual delivery following correction of postural abnormalities and reducing the size of emphysematous foetus by giving multiple incisions on foetal body saved 87% cases (Table 1).

Table 1 : **Incidence of various forms of dystocia in buffaloes- A record of 77 cases**

Maternal dystocias	Foetal dystocias	Treatment 77 (58+19[a]) (n=58)	Sex of the calf 77 (57+20[b]) (n=57)	Parity of dam 77 (63+14[c]) (n=63)
1. Hydroallantois 6.49% (5)[a]	1. Presentation position and postural abnormality 45.44% (35)	1. Caesarean section 43.10%(25) a. survived 72%(18) b. Died 28% (7)	1. Male 59.65% (34)	1. Ist calver 29.95% (19)
2. Insufficient dilatation of cervix 10.38% (8)	2. Monsters 7.79% (6)[b]	2. Manual removal 53.45%(33) (i) Survived 87%(29) (ii) Died 13% (4)	2. Female 40.35% (23)	2. 2nd Calver 23.81% (15)
3. Narrow pelvis 7.79% (6)	3. Macerated foetus 9.09% (7) [a,b,c]	3. Foetotomy 3.34% (2) (i) Survived 50%(1) (ii) Died 50%(1)		3. Pluriparous 46.03% (29)
4. Uterine Intertia 3.89% (3)	4. Mummified foetus 9.09% (7)[a,b,c]			
Total 28.57% (22)	71.43% (55)	58	57	63

a. Treated by hormonal therapy; b. Sex could not be known; c. Parity not recorded.

In all the cases , a suitable therapy was instituted to check the haemorrage, infection, shock and to prevent the post-treatment complication.

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Efficacy Of Isopropyl Alcohol As Epidural Anaesthetic In Bovine Obstetrics*

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ABSTRACT

Clinical evaluation of epidural administration of a local anaesthetic (Lignocaine or Bupivacaine) along with isopropyl alcohol was carried out in 10 obstetrical cases. Lignocaine worked well in fresh clinical cases which required less than one hour per vaginal manipulation. Those cases which required more than one hour for obstetrical manoeuvres, bupivacaine gave a fairly good caudal analgesia lasting two to four hours.

* * *

This study was envisaged to evaluate the prolongation of caudal epidural analgesia by a combination of either Lignocaine Hcl or Bupivacaine Hcl with isopropyl alcohol in obstetrical cases. Ten clinical cases presented to Veterinary Clinic, P.A.U., Ludhiana formed the subject of this study. The type of cases included for the study were dystokia (2 cases), prepartum vaginal prolapse (2 cases), postpartum prolapse (3 cases) and rectal/ rectovaginal prolapse (3 cases). Most of the cases except dystokia were handled before and subjected to the caudal, epidural analgesia by administering lignocaine HCl without any successful results.

These cases were given a combination of local anaesthetic (lignocaine HCl - 5-7 ml or bupivacaine HCl 6-10 ml) and after one hour 3 ml of isopropyl alcohol in sacro-coccygeal space. Pin prick method was used to know the

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loss of sensation of tail, perineum, inner aspect of thighs and around anus. The suspension of defecation, incoordination of hind limbs and any other complications, if any, were also recorded in each animal. No straining was reported for 10-14 days in four cases which included postpartum prolapse, prepartum prolapse and rectovaginal prolapse and were given lignocaine and isopropyl alcohol. One of the animal after 10 days again showed straining and the above treatment was again repeated. After that no straining was reported. In two cases of dystokia and one post-partum vaginal prolapse, administration of bupivacaine and isopropyl alcohol stopped the straining and the animals were discharged.

Administration of bupivacaine and alcohol in the four chronic cases having a history of severe straining included rectal prolapse, rectovaginal prolapse, postpartum and prepartum vaginal prolapse resulted in the control of the straining. Administration of lignocaine or bupivacaine followed by isopropyl alcohol undoubtedly prolonged the duration of caudal anaesthesia considerably and prevented staining for many days. The use of 45% alcohol in bovine obstetrics particularly in vaginal prolapse has been claimed to have evoked 44.4 to 100% response (Senze, 1963). Pain due to severe straining was successfully removed by injection of 22-25% ethyl alcohol in cows (Treusch, 1975). Administration of procaine hydrochloride epidurally has been observed to facilitate the replacement of the postpartum prolapse of uterus and it obviated straining and desensitized the perineum for suturing (Arthur, 1975).

Administration of lignocaine or bupivacaine with isopropyl alcohol resulted in sleepiness. Similar observations have been reported by Ritchie and Cochen (1970). A mild ataxia of the hind limbs, inability to move their tail in response to flies or birds was observed for many days. Ballooning of vagina observed in all the clinical cases, seem to have aided the retention of vagina after repositioning in clinical cases of vaginal prolapse. Similar observations have been reported following internal pudendal nerve block in cows (Deshmukh and Deshpande, 1980). Prolonged caudal epidural anaesthesia proved to be a good aid for the treatment of prolapse of genitalia.

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Changes In The Volume And Biochemical Constituents Of Caprine Foetal Fluids With Gestational Age

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ABSTRACT

The volume of foetal fluids and total protein, uric acid, creatinine, electrolytes viz. sodium, potassium, magnesium and inorganic phosphorus were estimated in the amniotic and allantoic fluids of local nondescript goats at different stages of gestation. Total protein concentration was higher in the allantoic fluid, compared to amniotic fluid. Uric acid and creatinine concentration increased progressively with advancing gestation. Sodium concentration was significantly higher in the amniotic fluid than in the allantoic fluid, whereas potassium and magnesium concentrations were higher in the allantoic fluid. There was no noteworthy difference in the inorganic phosphorus concentrations in the two fluids.

* * *

Bongso et al. (1979) observed that the caprine total foetal fluid volume had increased with gestation. Protein concentration of the foetal fluids is remarkably low as compared to that of the maternal plasma or foetal blood. Increasing accumulation of nitrogenous waste products in the foetal fluids may also be expected as a concomitant of accelarated protein metabolic turnover in the foetus during advanced stages of pregnancy.

In sheep, gestational changes in the electrolyte concentration of foetal fluids have been studied by Alexander *et al.* (1958), Mc Cance and Widdowson (1961) and Mellor and Slater (1970, 1971 and 1974). The work reported in this paper documents changes in the foetal fluid volume, total protein, uric acid, creatinine and electrolytes concentration at different stages of pregnancy in goats.

Materials and Methods

Pregnant goat uteri were collected from the Civil Slaughter House, Mhow. Immediately after slaughter the uteri were removed, chilled in ice and brought to the laboratory. These were promptly dissected to expose the amniotic and allantoic sacs, and those having single normal foetus were taken for the study. The amniotic and allantoic fluids were aspirated separately, using a 18 gauge hypodermic needle and 50 ml all glass syringe and measured in a measuring cylinder.

The gestational stages were designated as described by Kadu and Kaikini (1987). Briefly, Stage I, crown-rump length of foetus measuring 4 cm or less; Stage II, crown-rump length of foetus measuring 4 to 20 cm; Stage III, crown-rump length of foetus more than 20 cm. Corresponding to each gestational stage, the foetal fluids from six pregnant uteri were taken for investigation.

The total protein concentration was estimated by the method of Reinhold *et al.* (1950). The uric acid concentration was measured by the method of Henry *et al* (1957), and creatinine concentration was estimated by the method of Folin and Wu (1919). Sodium and potassium concentrations were determined by flame photometry, as outlined by Oser (1965) using a digital flame photometer. The magnesium concentration was estimated by the method of Andreason (1957). The Fiske and Subbarao (1925) procedure was employed for estimation of inorganic phosphorus.

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Results and Discussion

The amniotic fluid volume progressively increased from stage I to stage III of gestation. However, the allantoic fluid decreased in stage II followed by a marked increase in stage III of gestation (Table 1).

Table 1 : **Volume (ml) of goat foetal fluids (Av.± SE)**

Stage of gestation	Amniotic fluid	Allantoic fluids
I	44.66 ± 1.33	118.83 ± 7.33
II	237.50 ± 30.82	67.16 ± 12.88
III	610.00 ± 80.78	518.16 ± 74.02

Table 2 : **Concentration (Av. ± SE) of biochemical constituents in goat foetal fluids in different stages of gestation.**

Constituents	Stage of gestation	Amniotic fluid	Allantoic fluid
Total protein (mg/dl)	I	54.16 ± 3.27^{a}	440.00 ± 41.62^{b}
	II	75.00 ± 7.74^{a}	1010.00 ± 174.38^{c}
	III	310.00 ± 63.18^{a}	1173.33 ± 130.99^{c}
Uric acid (mg/dl)	I	00.28 ± 0.03^{a}	00.69 ± 00.02^{a}
	II	00.77 ± 0.16^{a}	05.41 ± 1.22^{b}
	III	01.02 ± 0.17^{a}	07.68 ± 1.95^{b}
Creatinine (mg/dl)	I	04.20 ± 1.05^{a}	15.33 ± 4.72^{a}
	II	03.50 ± 1.22^{a}	117.67 ± 34.44^{b}
	III	20.70 ± 6.08^{a}	118.33 ± 1.05^{b}
Sodium (mEq/l)	I	94.67c ± 1.29^{c}	60.50 ± 6.47^{b}
	II	100.33 ± 2.39^{c}	38.00 ± 2.73^{a}
	III	67.17 ± 10.43^{b}	36.33 ± 3.97^{a}
Potassium (mEq/l)	I	20.00 ± 1.16^{b}	10.67 ± 0.76^{a}
	II	14.50 ± 1.12^{ab}	32.33 ± 4.66^{c}
	III	15.33 ± 1.23^{ab}	48.00 ± 4.31^{d}
Magnesium (mEq/l)	I	00.22 ± 0.07^{a}	00.60 ± 0.02^{a}
	II	00.19 ± 0.09^{a}	02.11 ± 0.66^{b}
	III	00.53 ± 0.18^{ac}	04.30 ± 0.13^{a}
Inorganic phosphorus (mg/100 ml)	I	03.89 ± 0.09^{a}	03.05 ± 0.24^{a}
	II	03.08 ± 0.40^{a}	03.63 ± 0.69^{a}
	III	04.92 ± 0.76^{b}	05.83 ± 0.22^{b}

Values with different superscripts under the same subclass vary significantly ($P<0.01$).

The protein concentration of the amniotic fluid did not vary significantly with advancing pregnancy. The value increased significantly in stage II and remained elevated in stage III, in the allantoic fluid (Table 2). There was no noteworthy difference in the uric acid concentration during various gestational stages in the amniotic fluid, the concentration was significantly higher in stages II and III, as compared to stage I, in the allantoic fluid. No significant difference in creatinine concentration was observed in the amniotic fluid.

The sodium concentration was significantly higher in stages I and II as compared to stage III in amniotic fluid and in stage I in the allantoic fluid. The potassium concentration increased progressively with advancing pregnancy. Similar trend was observed for magnesium concentration. The inorganic phosphorus values were significantly higher in stage III in both the foetal fluids (Table 2).

The volume of foetal fluids recorded in this study, is in agreement with the reports of Bongso *et al.* (1979) and Kadu and Kaikini (1984). The total protein concentration is appreciably lower in the foetal fluids because of concentration gradient, the blood plasma may contribute protein to the fluids. The values of uric acid and creatinine concentration in the present study are in close agreement with those reported by Luthra *et al* (1987a) for goats and Mellor and Slater (1974) for sheep. Lack of exchange of solutes might explain the higher uric acid concentration in the allantoic fluid.

The electrolytes concentration in the present study agrees with the report of Luthra *et al.* (1987b). Maintenance of concentration gradients of electrolytes may be the outcome of active transport mechanisms (Mc Cance and Widdowson, 1961). Mellor and Slater (1971) suggest that in the sheep chorioallantois pump mechanisms may reduce the sodium concentration of the allantoic fluid concomitant with increase in the potassium concentration. Alterations in the sodium and potassium concentrations of the allantoic fluid, associated with the advancement of gestation might be regulated by foetal plasma corticosteroids (Mellor and Slater, 1972). Inorganic phosphorus, so accumulated, is apparently utilised for skeletal development and growth of the foetus (Symond *et al*, 1966).

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Post-Partum Oestrus In Muzaffarnagri Sheep And Its Crossbreds

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ABSTRACT

Analysis of variance revealed highly significant effect of genetic groups and seasons on post-partum oestrus. Frequency distribution revealed that higher percentage of ewes exhibited post-partum oestrus in Muzaffarnagri (32.03%) between 61-75 days, whereas, in both the crossbreds it was between 46-60 days. The proportion of ewes exhibiting post-partum oestrus within 30 days of parturition was very less.

* * *

Considerable variations have been observed in the occurrence of first post-partum oestrus in Indian breeds of sheep ranging from 17 to 300 days (Sahni and Roy, 1967; Taparia, 1972 and Rawal *et al*, 1986). The present paper deals with the occurrence of first post-partum oestrus in Muzaffarnagri sheep and its crossbreds with Dorset and Suffolk.

Materials and Methods

A total of 339 ewes comprising of 256 Muzaffarnagri (MM), 65 Dorset X Muzaffarnagri (DM) and 18 Suffolk X Muzaffarnagri (SM) ewes of different parity maintained under semi-intensive system of management at CIRG, Makhdoom were used to study the post-partum oestrus interval. Heat detection from 10th day onward of lambing till 90th day was done twice a day (Morning and Evening) with the help of vasectomized teaser ram and the ewes exhibiting oestrus were identified. On the basis of our previous experiments, two lambing seasons i.e. March-April (Season-I) and October-November (Season-II) were practiced in the farm. The data were pooled and analysed by standard statistical procedures described by Harvey (1975).

Results and Discussion

Analysis of variance (Table 1) revealed highly significant effect of seasons ($P<0.01$) on post- partum oestrous interval indicating the ewes lambing in season-II had shorter interval (48.86 ± 1.76 days) than those lambed in season-I (64.79 ± 1.87 days). Whiteman *et al.* (1968) while studying in Dorset, Dorset X Rambouillet and Rambouillet reported that the post-partum oestrus interval was 44 days in ewes lambed in autumn and 66 days in spring which is in line to this study.

Post-partum oestrus interval averaged 56.83±1.28 days (Table-2). The mean duration of post-partum oestrus interval was observed as 58.89 ± 0.88; 52.53 ± 1.78 and 59.06 ± 3.29 days in MM, DM and SM respectively. Similar results have been reported by various workers (Taparia, 1972; Rondon and Combellas, 1980 and Velarde, 1980). However, long post-partum oestrus interval ranging from 73.0 to 157.5 days was observed by Barker and Wiggins (1964) and Rawal *et al.* (1986) while Sahni and Roy (1967) found comparatively lower range of 23.9 to 41.6 days in Bikaneri sheep.

Least square analysis of variance (Table 1) revealed highly significant effect ($P<0.01$) of genetic groups on post-partum oestrus, indicating that post-partum oestrus period was shortest in DM and longest in SM. Barker and Wiggins (1964) observed significant difference in post-partum oestrus between Rambouillet and Dorset indicating low duration for Dorset and high for Rambouillet. However, Rondon and Combellas (1980) did not find any

significant difference between the two breeds.

The percentage of ewes showing post-partum oestrus interval within 90 days after lambing in the present study was observed as 64.6, 60.2 and 62.1% in MM, DM and SM ewes respectively. The present results fall within the range as reported by Barker and Wiggins (1964) and Hunter (1968). However, Sahni and Roy (1967) in Bikaneri and Hafez (1980) in Dorset reported comparatively high percentage of ewes exhibiting post-partum oestrus having shorter duration. In the present study none of the SM ewes evinced post-partum oestrus within 30 days. Similar results were obtained in South African Merino as reported in the Annual Report (1983) that none of the ewes exhibited oestrus within 35-40 days after lambing. Frequency of post-partum oestrus interval is shown in Fig-1.

From the above findings it is concluded that heat detection in post parturient sheep can be useful after one month of parturition and 60% sheep can be rebred within 90 days of parturition resulting into 50% increase in total lamb production per year in the flock as compared to only one lambing per year i.e. routine breeding.

Acknowledgement

Authors are thankful to Director, Central Institute for Research on Goats, Makhdoom for providing facilities.

Table 1 : **Least square analysis of variance for various factors affecting post-partum oestrus interval in Muzaffarnagri sheep and its crossbreds.**

Source	df	SS	MSS	G. Value
Season	1	7531.05	7531.05	38.51**
Genetic group	2	2032.46	1016.23	5.20**
Interaction	2	609.97	302.48	1.55
Error	333	65128.00	195.58	

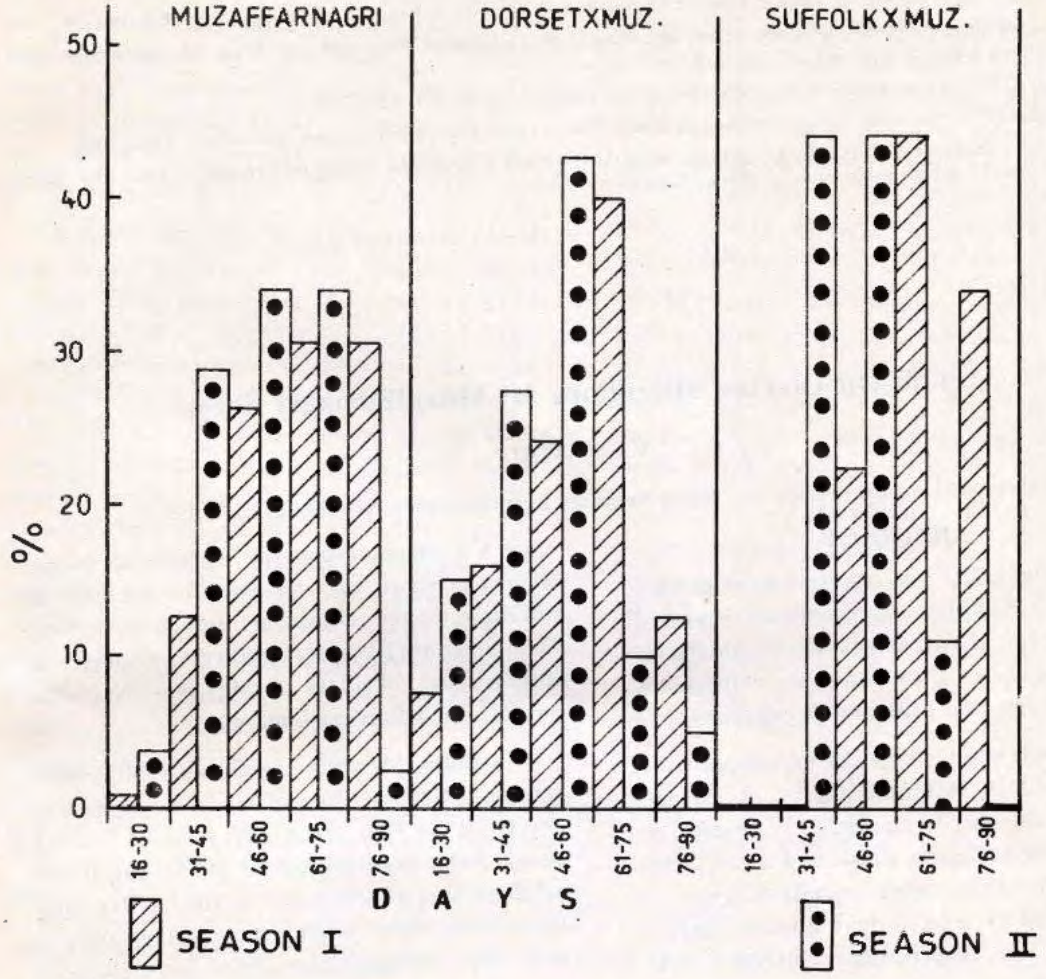
** P<0.01

Table 2 : **Least square means with their standard errors for various factors affecting post-partum oestrus.**

Factor and Sub-class	Post-partum oestrus (days)		
	Mean	S.E.	N
Overall mean (m)	56.83	1.284	339
Season of lambing			
Season-I	64.79 [a]	1.867	142
Season-II	48.86 [b]	1.762	197
Genetic group			
Muzaffarnagri	58.89 [a]	0.885	256
Dorset X Muz.ngri	52.53 [b]	1.783	65
Suffolk X Muz.ngri	59.06 [a]	3.296	18

Least square means connected with same superscripts do not differ significantly among each other within significant subclass.

Fig.1 POST PARTUM OESTRUS INTERVAL



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A Note On Uterine Microflora Of Muzaffarnagri Sheep

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ABSTRACT

Bacteriological investigtions were carried out on 60 samples. The isolates revealed Staphylococci, Escherichia coli, Corynebacterium pyogenes, Pseudomonas aeruginosa, proteus species and diphtheroid organisms.

However, similar types of organisms were also isolated in different phases of estrum, gestation and sterile animals. It would be difficult to establish a cause and effect relationship. Probably, these organisms exist in fewer number even in normal animals. Only a single case of Aspergillosis infection was observed.

* * *

Reports on microbial involvement of sheep uteri are scanty. However, few workers from abroad (Mikhailov *et al*, 1972; Mostafa *et al*, 1973; Adams, 1975 and Gamcik *et al.*, 1975) reported the presence of various microorganisms in the uterus of sheep. Most of the material was collected from slaughtered ewes with history of infertility. The most common organisms isolated were Pseudomonas sp., E. Coli, Corynebacterium sp., Streptococcus sp., Staphylococcus sp. and Bacillus species. In addition to the above mentioned microbes, Gamcik *et al* (1975) reported certain species of micrococus, Proteus vulgaris, Neisseria subflava and Sorcina lutea etc.

In India, Moorthy and Singh (1982) have reported bacterial flora of anterior vagina and cervix of 86 live sheep and uteri of 9 dead sheep. After examination, 82 of live sheep and all dead sheep were positive for bacteria and the isolates were Staph. aureus, Staph pyogenes, Str. zooepidemicus, Str. dysgalactiae, Corynebacterium renale, Cor. Ovis, Cor. equi, Cor. pyogenes, Listeria monocytogenes, E. Coli, Salmonella sp., Ps.vulgaris, Ps. aeruginosa, Brucella melitensis, Campylobacter fetus, venerealis and Clostridium welchii Type A etc.

In total 60 Muzaffarnagri sheep viz. 34 cyclic (on day 1, 3, 10 and 16 of oestrus cycle), 2 in 3 months pregnancy, 5 during 20-40 day post partum, 5 never evinced oestrus cycle, 6 sterile and 8 subfertile animals, were sacrificed.

Tissue samples from uteri were collected by taking all aseptic precautions. Individual tissue sample was triturated in sterile mortar and pestle and suspended in sterile saline. The suspended tissue was inoculated into nutrient broth, thiol broth, tryptose agar and blood agar for brucella and listeria and Sabouraud's agar for fungal isolation. All above media were incubated at 37°C for 24 hrs.

Nutrient agar, McConkey's agar and blood agar were inoculated from nutrient broth culture. These plates were incubated for 24 to 36 hours after inoculation at 37°C. The isolated colonies from different media were inoculated in peptone water and incubated at 37°C for 18 hours. These young cultures were used for biochemical tests e.g. MR, VP, indol, nitrate, citrate, urea, H_2S, gelatin, litmus milk, glucose, lactose, maltose mannitol, salicin, inocitol, sucrose, sorbitol, trehalose and dulcitol. These reagents were incubated at 37°C for 48 hours. Their fermentation reactions were read and on the basis of fermentation reaction all the bacterial isolates were typed.

Bacterial isolates from uterine tissue samples on the day of oestrum, 3rd, 10th and 16th day after oestrus were same in all above conditions. Bacterial isolates were *Staph. aureus, E. coli, C. Pyogenes, Ps. aeruginosa, Proteus.* Besides other micro-organisms the presence of these bacteria has also been reported by Mikhailov *et al.* (1972); Adams (1975); Gamcik *et al.* (1975) and Moorthy and Singh (1982). Pathogenic diphtheroids were also isolated from some samples.

The same type of organisms were also isolated from sterile, post-partum and pregnant ewes. There is no significant difference among type of bacteria recovered from these conditions. *A. fungus* and *A. fumigatus* were also isolated from only one case.

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Studies on Corpus Luteum Of Pregnancy In She-Camel *(Camelus dromedarius)* Throughout The Year

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ABSTRACT

146 genitalia of pregnant she-camel were collected. Pregnancy was recorded in all seasons and throughout the year. In all cases the left horn was found pregnant but in 58% cases the corpus luteum (CL) of pregnancy was observed on contralateral ovary. The contralateral pregnancy had 13.9% female and 44.3% male foetuses. The length, width and area of CL did not vary throughout the year. Season, sex of foetus and body measurement (curved crown-rump) did not affect size of CL of pregnancy in camel.

* * *

The presence of corpus luteum (CL) during normal oestrus cycle of camel is controversial (Abdo *et al*, 1968; Musa and Abusineina, 1978). CL has not been generally observed in ovaries of non-gravid camels except in some cases with occluded os-uteri (Shalash, 1965; Arthur *et al*, 1989). However, the CL is definitely present throughout the gestation period (Mukasa-Mugerva, 1981). Depending on the size, position and form of CL the shape of ovary during pregnancy is subjected to much variation. Shalash, (1965) and Nawito *et al*, (1967) reported variable size of CL depending on stage of pregnancy. In early pregnancy it has a flabby consistency, becoming larger and firmer as gestation advances.

Although literature is available on camel reproduction, investigation on different aspects of CL of pregnancy is much needed. In this study the influence of season, sex of foetus, body measurements of foetus (curved crown rump (CCR) and contralateral or ipsilateral presence of CL, on the size of CL were investigated.

Material and Methods

The genitalia of 146 pregnant she-camels were collected from Oursime and Cairo abattoirs. The date of service was unknown but none of them were in late stage of pregnancy. The collections wre done througout the year.

The curved crown rump (CCR) was measured from the face of head upto the tail of foetus. Length and breadth of CL were measured as per the method of Shalash (1965). Sex of foetus, site of pregnancy and side of ovarian activity during different seasons were also recorded.

Results and Discussion

Season and Pregnancy: Pregnant genitalia were available almost throughout the year indicating pregnancy in all seasons and months. However, availability of pregnant genitalia was more in April (32.55%), followed by July (15.11%). There was no variation in the length and width of CL during different months of year (Fig.1). Nawito *et al.* (1967) reported more pregnancy in the winter and spring than in summer and autumn.

Site of pregnancy and presence of CL: In all cases the left horn was found pregnant, but in 58% cases the CL was present on contralateral ovary. Nawito *et al.*(1967) and Mukasa-Mugerwa (1981) have reported that in 99.17% cases the pregnancy occurs in left horn. This may be possible due to the fact that more ovulations occur from left ovary

(Shalash, 1965 and Musa & Sineina, 1976). In contrast to this, during present investigation CL was found more on right side. However, more studies are needed to claim this point.

Sex of foetus and cross pregnancy: The length, breadth and their cross product of CL did not vary significantly with the ovary (right or left) or sex of foetus (Table 1). The CCR length also did not vary with change in ovarian activity or sex of foetus (Table 2). Cross pregnancy was maintained by a CL on opposite (right) side in 58.26% cases. Of these contralateral pregnancies, 13.91% were female and 44.34% males. Early embryonic migration is frequent among dromedaries camel (Shalash, 1965; Musa and Sineina, 1976; Mukasa-Mugerwa, 1981). Current observations indicate that more embryonic migration possibly occur in male foetuses. However, why pregnancy occurs only on left side is still not clear.

In three genitalia (1.74%) two CL were observed with only one foetus present. Musa and Sineina (1976) reported two (13.65%) to three (1.22%) with only one foetus. Hence probably some early embryonic mortality (EEM) could not be ruled out.

Left horn pregnancy was more common in camels with 58% cases, CL was contralateral. CL didnot vary in length and width throughout the year. Male foetuses were moe than female foetuses.

Table 1 : **Measurements of corpus luteum during different side of ovarian activity and with male or female fetus.**

Item	Length (L) (cms)	Width (W) (cms)	L x W (sq.cms)
Ovary			
Left (n=69)	2.25 ± 0.06	2.21 ± 0.05	5.30 ± 0.35
Right (n=77)	2.27 ± 0.06	2.17 ± 0.05	5.17 ± 0.18
Sex of foetus			
Male (n=97)	2.23 ± 0.05	2.15 ± 0.04	4.77 ± 0.15
Female (n=49)	2.30 ± 0.07	2.23 ± 0.06	5.25 ± 0.22

Table 2 : **Measurement of curved crown rump (CCR) length during different side of ovarian activity and sex of foetus.**

Ovary	Foetal CCR (cms)
Left (n=69)	62.987 ± 0.014
Right (N=77)	62.995 ± 0.014
Sex of foetus	
Male (n=97)	62.985 ± 0.012
Female (n=49)	62.996 ± 0.017

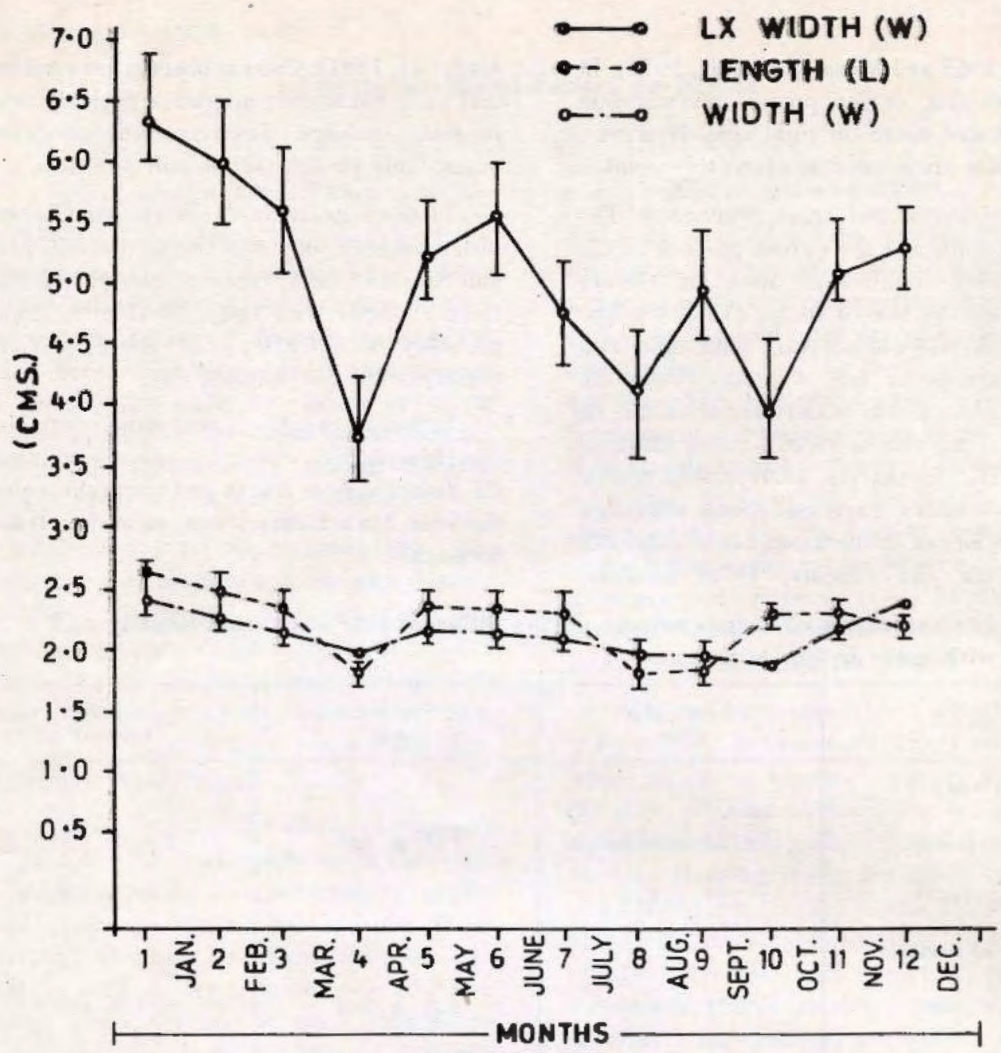

Fig. 1 : **Measurements of corpus luteum (CL) of Camel during the year at approx. same stage of gestation**

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Effects Of Gonadotrophic And Gonadal Hormones On The Genital Tract Of Bitch

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ABSTRACT

Thirty two sexually mature mongrel bitches (including eight controls), of nearly equal body weight were treated with exogenous gonadotrophic and gonadal hormones. The gonadotrophic hormone (PMS) failed to elicit any clinical response or gross changes in the ovary or genital tract, except a little vulval swelling and vaginal hyperaemia. Oestrus was induced when they were further given Vetestrol injections. Subsequent progesterone treatment maintained a pregnancy-like state of the genitalia when oxytocin was given to these animals. The clinical, gross and histological findings of the reproductive tract of different treatment groups were recorded and discussed.

* * *

The commonest form of infertility in the bitch is probably the functional disturbance of endocrine system which has not been explored to a great extent. Zondek (1931) and Scorgie (1939) established that hormones secreted by the anterior gland control the ovarian activity in mammals and initiate oestrus. Various workers attempted induction of oestrus in bitches with exogenous gonadotropin, oestrogen/progesterone and non-hormonal compounds (Leathem and Morrell, 1938; Willis, 1949; Teunissen, 1952). However, such oestrus induced cases were not accompanied with ovulation. The present study was undertaken to find out the effect of exogenous hormonal therapy on the oestrous cycle of bitch and to determine its effect on genitalia as revealed by clinical, gross and histological changes.

Materials and Methods

Thirty-two healthy, sexually mature, mongrel bitches 1½ to 2 years of age and 12-15 kg body weight were randomly divided into four groups of eight animals each. Two bitches in each group were treated as controls. At the commencement of the study, they were in anoestrus or in late metoestrus conditon with normal external genitalia.

The schedule of exogenous gonadotrophic and gonadal hormones administered in the 24 experimental bitches was so as to mimic oestrous cycle followed by pregnancy and parturition in normal live bitch. Eight control animals were injected with normal saline as placebo. The laparotomy of control and experimental animals of each group was performed on the same day (Table 1).

After the hormonal treatment, all the animals including controls in each group were observed for the clinical signs exhibited. Thereafter they were sacrificed on the schedule day and necropsy performed. The entire genitalia and gonads were collected, observed for gross changes in character and subjected to histological exmination.

Results and Discussions

In Group-1 bitches, no significant clinical

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Table 1 : **Schedule of Hormone treatment and clinical signs observed in bitches.**

Group	Bitch No.	PMS (Pregnyl inj. S/C Single dose	Interval of treatment	Stilbesterol (Vetestrol) inj. I/M.	Interval of treatment	Progesterone (Proluton) inj. I/M.	Interval of treatment	Oxytocin (Syntocinon) inj. I/M.	Laparatomy day	Clinical Signs			
										Vulval swelling	Vaginal Hyperaemia	Signs of Proestrus bleeding	Signs of Oestrus
I	1-6	500 i.u.	—	—	—	—	—	—	7	+	+	—	—
II	9-14*	500 i.u.	10 days	5 mg daily for 10 days	—	—	—	—	22	+++	+++	+++	+++
III	17-22	500 i.u.	10 days	5 mg daily for 10 days	10 days	50 mg daily for 60 days	—	—	92	+	+	—	—
IV	25-30	500 i.u.	10 days	5 mg daily for 10 days	10 days	50 mg daily for 60 days	2 days	10 i.u. at an interval of 2 hrs x 3 injs.	95	+	+++	—	—

* Bitch No.10 & 14 did not exhibit the above clinical signs

+++ = Well marked.

\+ = Slight.

– = No response

Table 2 : **Gross and histological changes in genitalia of experimental bitches following different hormonal treatments.**

Group	Gross changes of Genital Organs			Histological Changes of Uterus
	Ovary	Uterus	Cervix	
I	No gross enlargement. No prominentG.F.No.C.L. Several developmental follicles.	No visible enlargement. The horns were of uniform diameter.No change in luminal surface.	Cervix-uteri completely closed.	No marked changes.
II	Lobulated with presence of no. of follicles in various stages of development.	Enlarged and Oedematous.Slight enlargement of endometrial glands.	Cervix-uteri relaxed.	Endometrial epithelium increased in thickness.Mild glandular hyperplasia in all bitches.Stromal oedema,sub-epithelial erythrocytes extravasation.Macrophages scattered all over the endometrium.
III	Absence of mature follicles. C.L.absent.	Presence of abundant oedematous glands.Diameter of horns increased.	Cervix-uteri closed.	Glandular hyperplasia of the endometrium with infiltration of neutrophils. Vascular channels were more prominent. An increase in collagen fibre present in all layers of the uterine wall. Endometrial changes similar to normal met-oestrus.
IV	-Do-	Oedematous,patchy haemorrhagic spots.Engorgement of blood vessels.	Cervix-uteri relaxed	Increased vascularity of uterus.Myometrium showed interstitial oedema with diffuse lymphocytic infiltration.Myometrial vessels exclusively deleted and congested.Marked inflammation of stromal layer composed of neutrophils and lymphocytes.

response was observed following PMS treatment, except slight vulval swelling and congestion of vagina (Table 1) possibly due to lack in adequate oestrogen concentration in blood (Christee and Bell, 1971). All the external symptoms of oestrus were manifested following Stilbestrol injections in 16 out of 18 bitches under Group-2, 3 and 4 in about 6 days and the same was characterized by soft, red and enlarged vulva, highly hyperaemic vaginal m.m. and proestrus bleeding. The gross and histological changes of the genital organs of these animals resemble those of normal proestrous or oestrus states, with considerable oedema and stromal haemorrhage and mild glandular hyperplasia allied to the findings of Teunissen (1952) and Dow (1957). The two bitches that failed to respond, manifested mild vulval swelling without any proestrus bleeding.

Treatment with Progesterone for 60 days in 12 PMS and Stilbestrol primed bitches caused slight vulval swelling and vaginal hyperaemia. The uteri became oedematous, enlarged and mucous glands were abundant in the endometrium. The histological changes in the endometrium, post-progesterone therapy were identical to normal metoestrous condition. Group-4 bitches which received Oxytocin injections following Progesterone treatment, showed increased vascularity of the

uterus with patchy haemorrhage in subserous and sub-endometrial regions with thinning of myometrium simulating parturition. These findings are allied to those of Caldeyro-Barcia *et al* (1957) and McDonald (1980).

All the eight control animals were normal throughout and did not show any remarkable clinical, gross or histological changes.

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Dr. A. S. Kaikini
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CLINICAL ARTICLES

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Clinical Observations And Treatment Of Various Reproductive Disorders In Deoni Cows And Their Crossbreds

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Certain reproductive disorders like anoestrum, repeat breeding, dystokia, retention of placenta, cervico-vaginal prolapse jeoparadise the economics of production and reproduction. For achieving best reproductive efficiency in cattle the incidence of such disorders should be minimum (Pargaonkar and Bakshi, 1987). Present studies pertain to clinical observations and treatment of various reproductive disorders in Deoni and crossbred cows.

Materials and Methods

Gynaecological examination of 329 cows (94 Deoni + 235 crossbred) were carried out. Of these, 54 cows had various reproductive disorders thus: True anoestrum (8), Pre-partum cervicovaginal prolapse (9), retention of placenta (12) and post-partum septic metritis (25). These disorders were treated as under:

(1) *True anoestrum*: Improved feeding, roughages ad-lib, lucerne 3 Kg/day/cow, sugras (Dry Ration) 1 kg/day/cow, mineral mixture 1 kg/400 kg of feed. Treatment was carried out for 2 months.

(2) *Prepartum Cervico-vaginal prolapse*: Manual reposition of prolapsed mass and application of rope truss to vulva. Oxytetracycline I.M. @10 mg/kg body weight to avoid secondary complications.

(3) *Retention of placenta*: Manual removal of placenta alongwith intrauterine therapy with (i) Oxytetracycline I.V., @10 mg/kg body weight with Nitrofurazone 60 mg per bolus, 4 boluses per day I.U. for 3 successive days. (ii) Strepto-penicillin 2.5 gm + 10 ml distilled water I.U. for 3 days. (iii) Nitrofurazone 2% liquid 30 ml I.U. for 3 days.

The efficacy of drugs was confirmed by cessation of lochial discharge and completion of involution of uterus.

(4) *Post-partum septic metritis* was treated with (i) Streptopenicillin 2.5 gm + 7.5 ml distilled water I.U. for 3 days. (ii) Tetracycline HCl 2 gm bolus I.U. alongwith parenteral injection of streptopenicillin 2.5 gm + 7.5 ml distilled water for 3 days. (iii) Oxytetracycline: 10 mg/kg body wt. I.M. and Nitrofurazone 60 mg per bolus, 4 boluses per day inserted I.U. for 3 days. (iv) Nitrofurazone 2%, 30 ml I.U. for 3 days.

The efficacy of drugs was confirmed by cessation of pus and completion of involution of uterus.

Results and Discussion

The efficacy (%) of various treatments was found to be 62.5 for True anoestrum; 88.8 for pre-partum cervico-vaginal prolapse; 83.3, 50.0 and 50.0 for A, B and C treatments in cases of retention of placenta and 66.6, 100.0, 100.0 and 50.0 for A, B, C and D treatments used in treating the cases of post-partum septic metritis respectively. The drug efficacy against these reproductive disorders was encouraging. In cases of retention of placenta and post-partum septic metritis the response (%) obtained by treating cases with intrauterine therapy alone was lower than that reported

when combination of I.U. therapy alongwith parenteral injection of antibiotics was used. The difference in the response may be due to additional antibacterial coverage provided by the parenteral therapy.

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Clinical Trials With 'Receptal' For Treatment Of Delayed Ovulation In Cross-bred Cows

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Ovulatory defects in heifers and cows are major causes of infertility in cattle. In crossbred cows the magnitude of delayed ovulation is so high that it has become a limiting factor in the economic maintenance of dairy cattle. Systematic investigations carried out recently revealed a high incidence of delayed ovulation among crossbred cows in Kerala (Iyer *et al* 1992). Administration of hormones LH, HCG, and GnRH hasten the process of ovulation. Encouraging results have been reported with GnRH (Lee *et al* 1981., Nakao *et al* 1983). Administration of GnRH causes peak LH concentration within 30 to 60 minutes which resembles that of LH surge during late oestrus in normally cycling animals (Leslie, 1983).

Beneficial effects of administration of Receptal, an analogue of GnRH, were reported in anoestrum in buffaloes and postpartum cows (Pattabhiraman *et al*, 1986). Rao and Naidu (1987) observed that administration of 'Receptal' improved overall conception rate in crossbred cows. Under field conditions, the beneficial effects of 'Receptal' in the treatment of delayed ovulation in crossbred cows are reported in this paper.

Materials and Methods

40 crossbred cows with ovulatory disturbances due to delayed ovulation based on history and gynaeco-clinical examination were selected for the study. They were administered 2.5 ml of 'Receptal' I.M. at the time of insemination. The animals were re-examined 11 to 12 days after insemination and those having mature CL in either of the ovaries were considered to have ovulated. All the cows were examined for pregnancy at 45 to 60 days after A.I.

Results and Discussion

Clinical examination 11 to 12 days after administration of 'Receptal', revealed active CL in 36 out of 40 cows, indicating that ovulation had occurred in 90% of the cows. Pregnancy was confirmed in 28 out of 40 cows (70%), 45-60 days after A.I.

The significant response of the treated animals in respect of induction of ovulation

and subsequent pregnancy can be attributed to the onset of LH surge in late oestrum resulting in ovulation by Receptal therapy as per Leslie (1983). The difference between conception rate and the ovulation rate may be due to other contributing factors which result in failure of fertilization. These results indicate that administration of 'Receptal' is of great value in correcting delayed ovulation and ensuring better conception rate in crossbred cows under field conditions.

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Comparative Efficacy Of Intrauterine Iodine Infusions In Induction Of Oestrus In Anoestrus Cows

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In Veterinary Medicine Lugol's solution is extensively used since long, though irritant and painful. It is gradually being replaced by new preparations like Povidone which is non-irritant with similar action. The present work was undertaken to study the comparative efficacy of different iodine (1%) preparations alongwith the emperical Lugol's iodine solution.

36 Gir and cross-bred farm cows which did not exhibit oestrus upto 90 days post-partum were gynaeco-clinically examined. Their ovaries were smooth, hard, inactive, without any palpable CL or follicles. Cervix and uterine horns were atonic flaccid with pale vaginal m.m. The cows were apparently healthy and free from infection. These 36 anoestrus cows were grouped into 4 treatment groups. The drugs were infused with 50 ml syringe by passing the tip of A.I. pipette through external os for one-two folds of cervix.

Group-I comprised of 12 cows with 20 ml Lugol's iodine 1% infused intrauterine. Lugol's iodine was locally prepared with 5 gm Iodum +12 gm potassium iodide +100 ml distilled water. This was used as a stock solution which was further diluted to get 1%

solution. Group-II comprised of six cows in which 20 ml Pivipol (Povidone iodine 1% AR-EX laboratories Ltd., Bombay) 1% w/v was infused intrauterine. Group-III comprised of six cows, in which 20 ml of Betadine (Povidone Iodine 5% Wochardt Ltd., Bombay) 1% was infused after diluting with double glass distilled water. Group-IV was kept as control and comprised of 12 cows to which placebo was given intrauterine @20 ml.

After treatment the cows were critically watched for heat. Cows that did not respond, were given second dose after seven days. Maximum three such treatments were given at 7 days interval.

The cows which manifested heat symptoms within a span of three weeks after the last treatment were considered to have responded to the clinical trial. Cows were confirmed pregnant after six weeks following service or A.I.

In group-I, 3 (25%); group-II, 1 (16.7%) and group-III 2 (33.4%) cows manifested heat symptoms at an average of 22, 36, 22.5 days respectively. In control group (IV), 2 (16.7%) exhibited heat at an average of 24.5 days. The conception rate in the respective groups was 66.7%; 0.0%; 50% and nil in the control group.

The value of chi-square test (6.66 NS) indicated no significant difference in the treated and control groups. These findings are not comparable with those of Eramacemikov (1964), Glotra *et al.* (1969), Deopurkar (1974), Porwal *et al.* (1976) and Deshpande and Sane (1977). However, a similar poor response was reported by Swensson (1971) and Chauhan and Singh (1979).

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CASE REPORTS

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Testicular Calcification In A Haryana Bull

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Testicular calcification is the last stage of testicular degeneration following orchitis and fibrosis and 75% of the testicular pathology is related to this condition (Roberts, 1971). However, the reports on testicular calcification are scarce. This malady is reported in a breeding Haryana bull.

The seven years old bull was presented to the clinic for castration with history of scrotal injury few months ago. Thereafter, there was severe swelling (1½ times) with acute pain. Local veterinarian treated the animal with recovery within 20 days. After this, the cows mated to this bull were not settling to service. Hence, the owner wanted to use it for draught purposes after castration.

On clinical examination the animal was found healthy. On palpation the testes appeared to be shrunken in size, fibrotic in consistency and lacked tonicity or elasticity. The testes were removed by the open method of castration. Longitudinal sectioning showed calcified masses within its tissues (Fig.1). Radiographic examination also substantiated the calcification.

Testicular calcification has been reported in the testis of a Shorthorn yearling (Arthur *et al.* 1989) and a buffalo (Rao, 1982) bull. Roberts (1971) stated that acute trauma causing interference in circulation may cause rapid or progressive testicular degeneration in

Fig. 1: **The longitudinally opened testis of the Haryana bull showing calcification (arrow).**

males, as it occurred in the present case also. The reduction in the size of testes from normal depends on the duration and degree of atrophy of seminiferous tubules (Aman, 1960). Jubb *et al.* (1985) described that with the rapid but permanent degenerative process, fibrosis takes place and subsequently the connective tissue itself may become calcified. The examination of calcified testes radiographically (Barker, 1956) and for its tone or elasticity (Hahn *et al.*, 1969) are considered to be good tools in its diagnosis, as observed in the present case also.

Acknowledgement

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Foetal Mummification And Uterine Rupture In A Cross-bred Cow

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A seven year old Friesian x Hariana cow with the history of about 4 months over gestation was presented to the veterinary clinics. When the animal was at full term, it exhibited signs of abdominal pains for two days. Thereafter, gradually udder got shrunken, followed by resumption of feed and water which was stopped for time being. However the cow did not show signs of parturition.

Rectal (PR) examination revealed a doughy mass deep inside the abdominal cavity without any fluid in it and diagnosed to be a case of mummified foetus. However, just anterior to the cervix there was a gap between cervix and uterine body and muscle fibres could be palpated in this gap. Cervix could easily be lifted up PR, feeling no tension over it, and this suggested uterine rupture also.

As the case was very old and suspected for uterine rupture, caesarean was performed by giving 30 cm long incision about 5 cm lateral to the milk vein, by the standard method. On opening the abdominal cavity, uterus was found tightly adhered over the mummified foetus in the form of a round mass without any fluid in it. The uterine wall was incised and when foetus was removed by traction whole of the uterus alongwith the foetus adhered over it came out (Fig.1). Incised part of the uterus and site of rupture anterior to cervix did not show any bleeding. Foetus was fully developed with intact hair coat, stained with reddish brown colouration. Abdominal cavity was sutured back and suitable therapy was given to prevent any infection and post-operative complication. Animal recovered and was discharged from the Clinics.

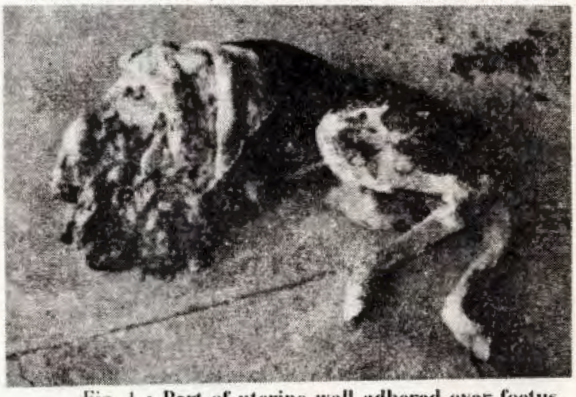

Fig. 1 : Part of uterine wall adhered over foetus.

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Dicephalic Monster In A Crossbred Cow

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A crossbred cow (50% H.F.x Deoni) age 5 yrs, belonging to University Cattle Project, Parbhani was presented for the treatment of dystokia. Gestation period was complete. There was no previous history of dystokia.

The cow was straining hard. Two forelimbs of the foetus were protruding out of vulva. P.V. examination indicated that there were two heads of the calf, which obliterated the birth canal. The calf was live. It was impossible to pull the calf only by applying traction to the forelimbs. Therefore eyehook threaded with cotton rope was applied to the lower mandible of right side head and traction was applied, which resulted in relieving the dystokia.

The calf was born live. It was a female calf with two heads joined together at the level of mandible. There were three ears, two muzzles, two mandibles and four eyes (Fig.1). The calf tried to stand for suckling the dam, but was unable to do so. Colostrum was artificially fed to the calf through nippled bottle. But the calf could not survive. It died after 24 hours.

On P.M. examination it was found that there was single larynx, trachea as well as oesophagus (Fig.2). All the visceral organs were normal without any duplication. Uterus and ovaries were small and rudimentry.

On scanning the breeding history, congenital cause, could not be trac[illegible].

Fig. 1 : Dicephalic Monster

Fig. 2 : Showing single larynx, traches and oesophagus on P.M. examination

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Recto-Uteral Fistula Leading To Delivery Per Rectum In A Murrah Buffalo

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Formation of recto-uteral fistula is very rare in bovine. Present report describes the recto-uteral fistula in a buffalo resulting in unusual delivery through rectum.

* * *

An 8 year old Murrah buffalo, with the history of per rectal delivery of the foetus was presented to the Clinics. As per owner, on gestation completion, buffalo attempted to parturate, but could not do so and kept on straining. After prolonged straining, owner observed foetal forelimbs at the anal opening instead of vulvar orifice and the foetus was removed by forced extraction. Examination revealed a greatly relaxed anal sphincter without any laceration. Cervix was not fully dilated and vaginal wall was intact. On P.R. examination, hand directly approached the ruptured uterus through about 10" long tear at the rectal floor, with faeces adhering over the uterine wall. General condition of the buffalo was poor.

After achieving epidural analgesia, rectum was evacuated with soft water anaema and the torn rectal floor was repaired per rectum with cadgut No.3 applying continuous sutures. To repair the uterus, left flank laparotomy was done. Blood clots and faeces smeared over visceral organs were removed and abdominal cavity was thoroughly lavaged with antibiotic solution. Uterine tear was sutured with cadgut No.3 using lambert technique and abdominal cavity closed. Inspite of heavy antibiotics and fluid therapy, the animal succumbed on second day of surgery.

Emphysematous foetus, dropsy of the foetal membrane and uterine torsion are important causes of uterine rupture near parturition (Roberts, 1986). In present case, violent uterine and abdominal muscle contractions with insufficiently dilated cervix, might have directed the foetal forelimbs upwards resulting in rupture of uterine wall and rectal floor. Severe peritonitis resulted in death of the dam since abdominal lavage, heavy antibiotics and fluid therapy proved to be ineffective in such extensive contamination of abdominal cavity with faeces. Krishnaswamy *et al.* (1981) also experienced discouraging results where foetal maceration resulted in uterine rupture, rectal perforation and peritonitis.

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Dicephalus Monster In a Buffalo Calf (Bubalus bubalis)

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Ten year old pregnant Murrah buffalo having fifth parity was presented to the Obstetrics & Gynaecology clinic on 20th October, 1991 with the history of dystokia. P.V. examination revealed the presence of live foetus in the birth canal. There was complete dilatation of cervix and the foetus was presented anteriorly with fully extended fore limbs. Double heads were palpated in the pelvic cavity and it was suspected to be a monster. The physical condition of the animal was normal, although she was straining. It was decided to deliver the foetus per vagina by mutation.

After giving epidural anaesthesia and proper lubrication with liquid paraffin, a long blunt hook was fixed in the inner canthus of right eye. A judicious traction was applied on the forelimbs and head by the assistants, while the operator directed the foetal delivery through the birth canal by manual operation. Double headed foetus was delivered alive. But soon after delivery, within fifteen minutes the foetus died. It was a male monster (double headed) with fusion of the neck at the middle of their length. The monster had a pair of forelimbs, a pair of hind limbs, single trunk and a tail, all being apparently normal (Fig.1).

The possibility of normal vaginal delivery of this monster might have been due to parous nature of the buffalo, apparently normal size of maternal pelvis and meticulous application of the obstetrical technique.

Fig. 1 : **Dicephalus monster**

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Foetal Mummification In Swine

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During examination of the gravid genitalia of slaughtered pigs, one specimen attracted our attention as only one foetus amongst all was of larger size (Plate 1). On opening both the uterine horns along the greater curvature, it was noticed that only one foetus had been normally developing while rest of the foetuses were mummified (Plate 2). Detailed observations recorded thereon are as follows:

1. *Ovaries* : (i) Right ovary (No. 11) presented 9 corpora lutea and one para ovarian cyst (No. 13). (ii) Left ovary (No. 12) presented two corpora lutea. Both the ovaries presented 11 corpora lutea corresponding to 10 foetuses indicating that one of the ovum might have been resorbed in the uterus without fertilization and development.

2. *Uterine horns* : (i) Right horn : Greater curvature was 179.0 cm while lesser curvature was 90.0 cm. Average circumference at the centre of six mummified foetuses was 11.72 cm. (ii) Left horn : Greater curvature was 196.0 cm while lesser curvature was 85.0 cm. Average circumference of three mummified foetuses was 12.17 cm and that of normally developing foetus was 20 cm.

3. *Body of the uterus* : Lenght 9.0 cm.

4. *Observations on foetuses* (Table 1) : The entire gravid genitalia weighed 2.5 kg. and the placenta of normal foetus weighed 52 gm. Other foetuses showed papyraceous mummification with foetal placenta shrivelled, dried and parchment like. The fluids were absorbed and the uterus was found to be contracted on foetuses. In normally growing foetus allantoic fluid was dark red in colour and measured 25.0 ml. Amniotic fluid was amber coloured and measured 32.0 ml. The spacing or positioning of the foetus in pigs occurs between, Day 13 to 17 of gestation (Roberts, 1971). In present case average space between two consecutive foetuses was found to be 8.2 cm.

The age of mummified foetuses estimated according to Hammond (1954) was 40 days while the age of normally growing foetus was 60 days. Present findings are in agreement with Roberts (1971) who reported that death of foetuses in swine occurs during 40 to 90 days of gestation and they are expelled at parturition alongwith the normal foetuses. Ponds *et al.* (1960) reported that single mummified foetuses in a litter are more common than two or more. Our findings, are exactly opposite, where out of 10 foetuses only one was normally growing and others were mummified. Hence, this may be the rarest case.

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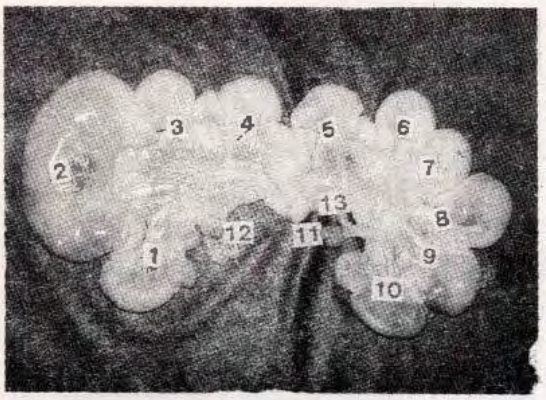

Plate 1 : **Gravid genitalia of pig showing larger size of only one foetus (No.2)**

Plate 2 : **Dissected gravid genitalia of pig showing normally developing foetus (No.2) and mummified foetuses (Nos. 1, 3, 4, 5, 6, 7, 8, 9 and 10)**

Table 1 : Observations on Normal and Mummified Foetuses in Pigs.

Sr. No.	Identification No. of foetus	Horn of uterus	Status of foetus	Sex	Heart Girth (cm)	CVRT length (cm)	Weight (gm)	Space between two consecutive foetuses (cm)
1	1	Left	Mummified	Female	6.0	19.0	33.0	1-2 foetuses - 8.0
2	3	Left	– " –	Male	7.0	19.0	26.0	2-3 foetuses - 9.0
3	4	Left	– " –	Male	6.5	16.0	27.0	3-4 foetuses - 8.5
4	5	Right	– " –	Female	5.5	17.0	40.0	4th foetus to body of uterus (left) - 8.5
5	6	Right	– " –	Female	5.5	19.5	32.0	Body of uterus (right) to 5th foetus- 8.5
6	7	Right	– " –	Male	6.0	20.0	52.0	5-6 foetuses - 8.0
7	8	Right	– " –	Male	7.5	23.0	63.0	6-7 foetuses - 7.5
8	9	Right	– " –	Male	8.0	22.0	52.0	7-8 foetuses - 8.0
9	10	Right	– " –	Female	7.0	21.0	53.0	8-9 foetuses - 8.4
								9-10 foetuses - 8.5
Average figures (n=9)					6.5	19.6	42.0	8.2
10	2	Left	Normal	Male	14.0	38.0	299.5	1-2 foetuses - 8.0

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Foetal Anasarca In A Doberman Bitch

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Various congenital abnormalities in animals have been described by Roberts (1971). This communication reports a case of foetal anasarca in Doberman bitch.

A two year old Doberman bitch was presented at college O.P.D. with the history that she was mated with a normal Doberman male 65 days earlier. Milk was dribbling from the teats and only slight labour was evident.After 24 hrs.a normal still born pup was delivered and therafter no labour was experienced. Failing to respond to Oxytocin injection, it was decided to undertake caesarian section.

A large sized oedematous pup (Fig.1) weighing about 1.8 kg was extracted. The length of pup(from head to tip of tail) was 18".Head was large,whereas, the jaws were extremely short with absence of eye balls in bony orbits with very short and erect ears.

Another extracted oedematous pup (Fig.1) though similar in appearance, was smaller in size (weighing only 0.5 kg and 12" in length).

The postmortem examination of both these edematous pups revealed that all internal organs were normal.

Factors responsible for these abnormalties are genetic or environmental and sometimes interaction of these two (Leipold *et al*,1983). Recessive autosomal characters are responsible for causing foetal anasarca in cattle.(Donald *et .al*.,1952).

Fig. 1 : **Foetal anasarca in Pups**

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