FERTILITY MANAGEMENT OF EARLY POST - PARTUM COWS WITH GONADOTROPHIN RELEASING HORMONE AND PROSTAGLANDIN F_2 ALPHA

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "**FERTILITY MANAGEMENT OF EARLY POST-PARTUM COWS WITH GONADOTROPHIN RELEASING HORMONE AND PROSTAGLANDIN F_2 ALPHA**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled "**FERTILITY MANAGEMENT OF EARLY POST-PARTUM COWS WITH GONADOTROPHIN RELEASING HORMONE AND PROSTAGLANDIN F_2 ALPHA**" is a record of research work done independently by **Dr. C. Jayakumar**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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We, the undersigned members of the Advisory Committee of **Dr. C. Jayakumar**, a candidate for the degree of Master of Veterinary Science in Animal Reproduction, agree that the thesis entitled **"FERTILITY MANAGEMENT OF EARLY POST-PARTUM COWS WITH GONADOTROPHIN RELEASING HORMONE AND PROSTAGLANDIN F_2 ALPHA"** may be submitted by Dr. C. Jayakumar, in partial fulfilment of the requirement for the degree.

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C. JAYAKUMAR

Dedicated to the

Memory of my Father

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Introduction

INTRODUCTION

Reproduction is the most critical component of profitable dairy industry. Although biotechnological advances have made it possible to improve bovine reproductive efficiency, attempts to reduce the calving interval to achieve the target of one-lactation per cow per year have not been fully achieved. The most common cause for this is the delay in the resumption of cyclic activity after calving.

Parturition is followed by an indefinite period of sexual inactivity - the post partum anoestrus. From an economic point of view, long post partum anoestrus has deleterious effect and this has made the physiologists and endocrinologists to study the factors influencing the reproductive system following parturition. The hormonal balance established to support pregnancy and its termination after parturition to reestablish the normal oestrous cycle is to be readjusted. The time required for this individual readjustment is quite variable and appeared to be influenced by endocrine, neurocrine, social, climatic, nutritional and genetic factors.

Occurrence of oestrus in the early postpartum period is the key factor which determines subsequent fertility as this depends on interplay of various endocrine events. Luteinizing hormone pulses in the early post partum period occurs at the rate of one in 4 to 6 hours. Ovulation of the dominant follicle can occur when luteinizing hormone pulses take place at the rate of one in every 40 to 60 minutes to stimulate maximum oestradiol production, positive feedback and an ovulatory surge of Luteinizing hormone (LH) and follicle stimulating hormone (FSH). Thus, FSH is mainly responsible for recruitment and selection of the dominant follicle. Exposure of an oestrogen active dominant follicle to frequent LH pulses is key to final maturation and ovulation. Factors that suppress LH frequency in the postpartum period delays onset of oestrus (Roche *et al.*, 1992). The short luteal phases observed in postpartum cows might be related to the contemporary involutive status of the uterus (Schirar and Martinet, 1982).

Postaglandin F_2 alpha influences the contraction of uterine smooth muscles and consequently helps in cleansing the uterine lumen of residual products resulting from parturition and increase the uterine tone. A rise in the level of prostaglandin metabolite (PGFM), (13,14-dihydro-15 keto prostaglandin F_2 alpha) occurred before or in conjunction with prepartum luteolysis and remained high during parturition. This returned to baseline values 10 to 20 days after delivery (Edqvist *et al.*, 1981). The higher concentration of PGF_2 alpha during early postpartum period was associated with both a faster rate of uterine involution and a stimulatory effect on follicular and luteal components of the ovary (Thatcher *et al.*, 1982).

The environmental factors particularly plane of nutrition, suckling and milk yield during the postpartum period were also mediated by the endocrine system (Peters and Lamming, 1984). The feasibility of hormonal therapy as a prophylactic measure for improvement of postpartum fertility, would be of great advantage particularly in Indian cattle, where husbandry and nutrition are often marginally adequate.

The present work was, therefore, undertaken to assess the effect of Gonadotrophin Releasing Hormone and Prostaglandin F_2 alpha administration during early postpartum period in reducing the service period by early resumption of oestrous cycle and establishing fertility.

Review of Literature

REVIEW OF LITERATURE

2.1 Postpartum period

2.1.1 Introduction

Reproductive efficiency of post partum cow has definite influence on calving interval and thus influences the future production potential and culling rate of dairy herd. Speicher and Meadows (1967) observed that extension of calving interval from 12 to 14 months resulted in an average reduction of 8.8 per cent in the annual financial return. Britt (1974) reported that with an acceptable rate of reproductive efficiency and with current management practices, a calving interval of 12 months or less can only be achieved by shortening the interval of first insemination to an average of 50 to 60 days postpartum. Holman *et al.* (1984) also reported that in lactating cows a 12 to 13 month calving interval was considered optimal under most management systems. A herd average calving interval of 12 months requires that cows conceive by 85 days postpartum. The postpartum period of the cow begins at parturition and continues until uterine involution is complete and normal oestrous cycles and oestrous behaviour resume (Paisley *et al.*, 1986). The postpartum period is often lengthened as a result of delayed involution of the uterus, delayed resumption of oestrous cycles or both which result in long calving intervals. Fetrow and Blanchard (1987) reported economic loss ranging from \$0.25 to 4.68 per day for cows not pregnant beyond 85 d postpartum. White *et al.* (1996) estimated a value of \$3 each day for cows open beyond 100 days.

2.1.2 Hormonal status during prepartum and postpartum period

Estrogens such as estrone (E_1), estradiol-17 alpha (E_2-17 alpha), estrone sulphate (E_1SO_4), estradiol-17 alpha sulphate (E_2SO_4), bovine placental lactogen and chorionic gonadotrophin like substance were identified as being produced by the bovine placenta (Bolander *et al.*, 1976; Hoffman *et al.*, 1976; Beckers *et al.*, 1980 and Shemesh, 1980). Edqvist *et al.* (1978) reported that just prior to parturition, final regression of the corpus luteum was associated with increase in PGF_2 alpha as monitored by high concentrations of PGFM in peripheral plasma. Appreciable data exist regarding the dynamics of oestrogen synthesis and secretion by the bovine placenta. Bovine placentome was the major site of oestrogen synthesis near term and estrone and E_2-17 alpha were the major oestrogens produced. Although, E_2SO_4 was the predominant oestrogen in the foetal compartment, E_1SO_4 was the major oestrogen in the maternal circulation (Hoffman *et al.*, 1979). Concentration of E_1SO_4 in plasma of the maternal unit increased at about 100 days of gestation (Eley *et al.*, 1979) and underwent a further increase during the last 40 days of gestation to maximal values just prior to parturition (Thatcher *et al.*, 1980; Collier *et al.*, 1981). Synergistic actions of oestrogens and corticosteroids were involved in the final induction of luteolysis and parturition and that the action of PGF_2 alpha on the myometrium was dependent on an environment containing low progesterone and high oestrogen levels (Hashmat and Shebata, 1983). It was also reported that plasma progesterone level decreased a day before parturition from the normal to 3.09 ± 1.74 ng/ml on the day of parturition and to 1.50 ± 1.20 ng/ml on the day after. Progesterone levels during gestation may differ in cows fed different levels of supplemental feed (Mobley *et al.*, 1984) and in those which had pathological conditions following parturition (Vlanov, 1984). LH pulses were seen in the first two months of pregnancy after which pulse frequency was reduced and almost vanished completely at the end of gestation resulting in low average LH concentration. Unlike LH, mean basal and maximum FSH concentrations as well as pulse frequencies were fairly constant throughout gestation (Schallenberger *et al.*, 1985).

According to Lindsay (1966) progesterone secretion during postpartum period was a prerequisite for oestrus expression. Robertson (1972), Corah *et al.* (1974) and Webb *et al.* (1980) indicated a transient elevation of plasma progesterone level in the absence of any detectable corpus luteum in dairy cows as well as in beef cows prior to the resumption of a normal ovarian cycle. Although, the origin of this transient increase in progesterone was not determined, it might originate from luteinised follicles, short lived corpora lutea or atretic normal or large cystic follicles and might be an expression of functional imbalance between the ovaries and the uterus (Schirar and Martinet, 1982).

Schams *et al.* (1978) observed a pronounced FSH fluctuation and irregular FSH peaks, independent of the onset of ovarian cycles prior to occurrence of postpartum ovarian cyclicity. Discontinuous release of FSH and LH occurred as early as day 4 postpartum in dairy cows, which increased to a frequency of about one pulse per hour 11 to 32 days postpartum (Foster *et al.*, 1980). Thereafter, levels of FSH rose first followed at a variable period by increases in basal levels of plasma LH. Lamming *et al.* (1981) remarked that, once an increased level of FSH had occurred at an early stage in the postpartum milked cow, differences in plasma FSH were not considered a limiting factor to the onset of ovarian activity. In spring-calved suckled beef cows, there appeared to be a depression in plasma FSH which contributed to the extended period of ovarian inactivity (Riley, 1982). According to Schallenberger (1985), FSH concentrations were low for a short period after calving, then rose and it was presumed that increase of FSH initiates follicular recruitment and selection of dominant follicle.

Webb *et al.* (1980) and Lamming *et al.* (1981) remarked that immediately before the first preovulatory LH surge, there was an increase in frequency and peak of LH episodes probably related to increase in pituitary sensitivity to endogenous gonadotrophin releasing hormone and to more frequent episodes of endogenous GnRH release. They further observed that in this respect, the pattern of LH release mimicked that observed during the three days prior to the preovulatory LH surge of the normal cycle. Peters *et al.* (1981) observed an LH pulse frequency between 0.25 to 1.25 pulses per hour in the postpartum dairy cow commencing several days before ovulation with pulse frequency negatively correlated with time to first ovulation. This pattern of LH release was important for ovulation and normal luteal function to occur. According to Nett (1987), anterior pituitary had a decreased content of gonadotrophins in late pregnancy and early postpartum period which was due to strong negative feedback of high oestrogen concentration in late pregnancy. According to Roche *et al.* (1992), the main events determining ovulation were the presence and exposure of a dominant follicle to the correct LH pulse frequency and remarked that inadequate LH pulse frequency resulted in low production of androgens in the follicle, and thus the pro-oestrous oestradiol rise did not occur, and the dominant follicle which was in the final stages of terminal differentiation underwent atresia.

Roche *et al.* (1992) opined that FSH was mainly responsible for recruitment and selection of dominant follicle and the exposure of an oestrogen active dominant follicle to frequent LH pulses was the key to final maturation and ovulation of dominant follicle. Ovulation of the dominant follicle occurred when an LH pulse occurred every 40 to 60 minutes to stimulate maximum oestradiol production, positive feedback and an ovulatory surge of LH.

2.1.3 Ovarian status

Perusal of literature revealed varying information with respect to follicular activity during postpartum period. Labhsetwar *et al.* (1964) reported that pituitary FSH content was higher on day one postpartum than on days 10, 20 and 30 postpartum. They also observed that mature follicles were present by day 21 postpartum. Morrow *et al.* (1966) was able to detect by palpation ovarian follicles 5 to 10 mm in diameter as early as 4 or 5 days postpartum. Morrow *et al.* (1966) and Wagner (1968) reported that around day 15 postpartum, follicles ready to ovulate were present. Morrow *et al.* (1968) also observed that the first postpartum ovulation occurred most often in the ovary opposite to that containing the corpora lutea of pregnancy (62 per cent) but with time the situation reversed so that in the 20 to 60 day period, 62 per cent of the ovulations took place on the side of the preceding pregnancy. He emphasised that normal cows had two ovulations during the first 30 to 35 days after parturition. Saiduddin *et al.* (1968) observed very little ovarian activity on the day after calving but considerable activity by day 10 postpartum. They also observed that the number of follicles were lowest on day 1 and highest on day 10 postpartum. Marion and Gier (1968) recorded that first ovulation after parturition on 13, 14 and 15 days in three production groups of dairy cows. Callahan *et al.* (1971) remarked that after day 10 postpartum, the ovaries increased in size and weight. Kesler *et al.* (1978) was able to detect ovarian follicles around 10 mm in diameter by day 4 or 5 postpartum. Dobson (1978) and Webb *et al.* (1980) opined that renewed follicular development on days 4 or 5 postpartum was due to the release of FSH. Schirar and Martinet (1982) and Savio *et al.* (1990) opined that the fate of follicles during early postpartum period was to undergo atresia because ovulation occurred only sporadically. Roche *et al.* (1992) reported that although follicles ready to ovulate were detectable around day 15 postpartum, factors that suppress LH pulse frequency delayed first ovulation.

2.1.4 Uterine status

Estimates of the average interval from parturition until completion of involution vary widely. Casida and Wisnicky (1950) reported that involution of uterus was complete in 29.4 days in dairy cows and that parity of the cow influenced the duration of involution period. According to Rasbech (1950) normal uterine involution was completed by day 25, and outlined a four stage sequence in involution. During the first stage, 1 to 8 days, vagina 8 centimetre band, cervix distinguishable by day 4 or 5 from the uterus and located at pelvic brim, uterine surface felt hard and corrugated. During the second stage, 8 to 10 days, entire uterine surface was palpable, smooth and soft feeling of post gravid horn, caruncles detectable as hazel-nut shaped processes, cervix hard and within pelvic cavity. During the third stage, 10 to 18 days, uterus a soft plastic body, caruncles and fluctuations less pronounced, cervix diminished in size and felt very firm. During the final stage, 18 to 25 days, uterine tone increased and post gravid horn similar to non-gravid horn. In Holstein Friesian cows, Buch *et al.* (1955) recorded 47 and 50 days for involution of uterus in normal and abnormal calvers respectively. Menge *et al.* (1962) reported 42.3 days for involution in the same breed. Morrow *et al.* (1966) reported that decline in uterine volume was irregular with a slow initial decrease from 4 to 9 days, and a period of accelerated decline to 14 days, followed by a slow and even reduction to 25 days. It was also revealed that the rate of involution decreased as the number of lactations increased. Tennant *et al.* (1967) concluded that decreases in uterine volume occurred rapidly between 10 and 20 days with only minor reduction thereafter. Marion *et al.* (1968) reported 39 days as the average interval from calving to involution and remarked that primipara took 34 days in contrast to pluripara which took 40.6 days. Gier and Marion (1968) found that the length of the gravid uterus was reduced to half of its gravid size in 15 days and complete involution occurred by 50 days after calving. Morrow *et al.* (1969) observed that the gravid horn remained slightly thickened and never involuted to pregravid stage. They indicated an interval ranging from 37.7 to 56 days for beef cows and an interval of 26.2 to 47 days for dairy cows. Wagner and Hansel (1969) reported that regeneration of uterine epithelium occurred by day 8 postpartum in the inter caruncular spaces in the areas which were not seriously damaged and complete restoration of the mucosa with endometrial glands took about 4 to 5 weeks. Francis and Raja (1971) reported that involution was complete within 32 to 44 days with a mean of 36.27 ± 0.69 days in Sindhi cows. Roberts (1971) recorded less time (26 to 52 days) in dairy than beef cattle (38-56 days) for involution and observed a longer involution period in pluripara than primipara. Choudhury *et al.* (1974) reported that primipara took 50.00 ± 1.67 days with a range of 28 to 77 days for complete involution. Jana and Mishra (1978) recorded an interval of 34.89 ± 4.00 and 23.91 ± 0.30 days from calving to the involution of gravid and nongravid uterine horns respectively in crossbred cows with normal calvings. The involution period was less in primiparous cows (32.61 ± 0.49 days) than pluriparous cows (36.94 ± 0.52 days). Nair (1979) reported 28.70 ± 0.60 and 37.15 ± 1.09 days for involution in normal and abnormal calvers of crossbred cows respectively. The duration was less in primiparous than pluriparous cows. The process of involution was rapid in young Siberian black pied cows than older ones with an average of 28.88 ± 1.9 days (Avdeenko, 1979). Lindell *et al.* (1982) speculated that uterine synthesis of PGF_2 alpha increased uterine tone which promoted involution and they associated long involution times with insufficient synthesis of prostaglandin F_2 alpha. Ruesse (1982) opined that after expulsion of the fetus, contraction process in the myometrium was sustained in order to expel the after birth and to maintain a small sized lumen of the uterus which hindered infections. Bhaskaran *et al.* (1983) reported an interval of 35.02 ± 0.78 days for uterine involution in normal calvers.

2.1.5 Correlation between uterine involution and ovarian activity

Buch *et al.* (1955) found that uterine involution and ovarian activity are interrelated. They observed that of all the cows that ovulated within 30 days postpartum only 6 per cent had uninvoluted uteri. But Menge *et al.* (1962) and Wagner and Hansel (1969) remarked that the process of uterine involution and ovarian activity were quite independent of each other.

Morrow *et al.* (1966) reported that the onset of postparturient ovarian activity and ovulation did not appear to be directly related to the rate of uterine involution although some conditions of the uterus could delay involution and the recommencement of oestrual activity. Foote (1971) observed that the involuting uterus did not influence pituitary gonadotrophin production and/or release during the postpartum period. Lauderdale (1972) and Anderson (1973) stated that the involuting uterus was involved in shortening of the luteal phase in many species, probably through the release of PGF_2 alpha which reached the adjacent ovary. Hartigan *et al.* (1974) observed faster rate of uterine involution in the non-gravid horn as short lived corpora lutea were more likely to occur on the side of previously gravid horn. It was also found that corpora lutea formed after the first ovulation, before 40 days postpartum were more likely to develop in ovary which did not carry the corpus luteum of pregnancy. Roberts and Mc Cracken (1976) and Chan (1980) reported that oxytocin, induced the release of PGF_2 alpha through its action on uterine muscle fibres and that this oxytocin induced PGF_2 alpha release might be responsible for the short life span of the first corpora lutea after calving in suckled animals. Quayam (1984) reported the occurrence of palpable corpus luteum in the ovary before day 20 postpartum prior to complete uterine involution.

2.2 Response to exogenous hormones

2.2.1 Gonadotrophin releasing hormones

Exogenous administration of gonadtrophin releasing hormone and its analogues have been reported to have a beneficial effect on early induction of postpartum oestrus (Schams *et al.*, 1973). GnRH injection in cattle induced release of LH and FSH (Kattenbach *et al.*, 1973; Britt, 1974; Schams *et al.*, 1974; Kesler *et al.*, 1977). Cummins *et al.* (1975) opined that treatment of cows with GnRH resulted in LH release similar in magnitude to that found at normal oestrus. According to Britt *et al.* (1977), there was a reduction in infertility and reproductive disorders after treatment with GnRH in early postpartum as a prophylactic measure. But Webb *et al.* (1977) found that LH release in response to GnRH was abrupt and biphasic and failed to induce normal ovarian cyclicity in animals with reproductive disorders. According to Fernandes *et al.* (1978) LH release in response to GnRH treatment was not restored fully until after 10 days postpartum. Foster *et al.* (1980) and Peters and Lamming (1984) found that the LH response to GnRH injection was significantly less in cows injected on or before day 5 postpartum than in cows injected on days 7 to 10 postpartum.

Attempts to induce ovulation in postpartum cows by single intramuscular injection of 100 to 500 μg GnRH (Schams *et al.*, 1973; Lamming and Bulman, 1976; Garverick *et al.*, 1980; Aboul-Ela and El-Keraby, 1986) gave variable results. At the above dose levels LH release of preovulatory surge magnitude usually occurred depending on the responsiveness of the pituitary. GnRH induced ovulation in postpartum dairy cows that had large follicles (≥15 mm) at the time of GnRH treatment (Garverick *et al.*, 1980). Lofstedt *et al.* (1981) infused GnRH at the rate of 15 ug/hour intravenously for 12 hours in postpartum beef cows and found that preovulatory type LH surge occurred in all cows within 13 minutes of the start of infusion, but only 4 out of 14 animals ovulated.

Britt *et al.* (1977) reported that when 200 μg GnRH was given by intramuscular route at 8 to 23 days after parturition, the frequency of ovarian follicular cysts reduced considerably with that of control. Although the reproductive efficiency did not differ among control or GnRH treated cows in the herd, there was a reduction in the infertility and reproductive disorders in the early postpartum dairy cows given GnRH. Nash *et al.* (1980) found that a single intramuscular injection of 250 μg GnRH two weeks after calving resulted in a better calving to conception rate (74.5 vs 56.0%), better overall conception rate (70.6 vs 51.1%) and a lower number of services per conception (1.23 vs 1.74) than those of control. Zaied *et al.* (1980) pointed out that GnRH treatment as early as 12-14 days postpartum, could initiate cyclic ovarian activity and thus could be useful in reducing abnormal ovarian activity. However, they pointed out that an elevated preinjection concentration of oestradiol-17ß and follicular growth were important for GnRH induced ovulation. Bostedt and Maurer (1982) reported that injection of GnRH between tenth and twelfth day postpartum resulted in a reduction of disturbances in the growth of follicles.

Benmrad and Stevenson (1986) observed that treatment with 200 μg GnRH between day 10 and 14 postpartum, reduced intervals to first ovulation and first detected oestrus as well as increased the proportion of cows with three or more ovulations before first service. Pattabiraman *et al.* (1986) recorded that when early postpartum buffaloes and cows were treated with 5 ml Receptal, eighty per cent of buffaloes and sixty per cent of cows showed normal ovulatory oestrus on an average 20.6 and 16.7 days after treatment. According to Archbald (1990), administration of 100 μg GnRH 14 days after calving reduced the days open and services required per conception than the control group. According to Roche *et al.* (1992), GnRH administration in early postpartum period could ovulate the first dominant follicle but there was a high incidence of short silent cycles. Crowe *et al.* (1993) observed that single injection of GnRH analogue during the growing plateau or declining phase of the first postpartum dominant follicle of suckler cows, induced ovulation but did not alter the proportion of cows with short cycles. However, Heuwieser *et al.* (1994) stated that the efficacy of GnRH in early postpartum might not be consistent in all parities and body condition groups. They found decreased conception rate in cows with first lactation but beneficial for cows of second and higher lactation groups. Palta and Madan (1995) suggested that pituitary responsiveness to GnRH did not appear to be a limiting factor for resumption of oestrous cycle in early postpartum period.

Fernandes *et al.* (1978) correlated degree of LH response to GnRH with plasma oestradiol concentration. Langley and O'Farrell (1980) observed no difference in the conception rate of cows with or without GnRH injection after normal or disturbed parturition as long as the expulsion of the placenta had occurred in due time. Bostedt and Maurer (1982) proved that the uterine involution after a GnRH injection occurred quicker in cows with retained placenta whereas no difference in uterine involution could be noticed in cows without retention of placenta. They further opined that many cows with retained placenta showed abnormal ovarian function as determined by rectal palpation and when GnRH was injected on days 10 and 12, ovarian function on day 30 appeared normal whereas control cows still had ovarian abnormalities. Leslie *et al.* (1984) opined that administration of GnRH in cows with retained placenta produced improvements in certain parameters of reproductive performance, provided early postpartum breeding was practiced. This was confirmed by Chen *et al.* (1986) and Wildeus *et al.* (1987). Bosu *et al.* (1988) and Mori *et al.* (1988) reported specifically a positive effect of GnRH in cows with retained foetal membranes after treatment with GnRH in the second week postpartum. White *et al.* (1996) observed that when cows that had undergone difficult calvings were given 100 μg GnRH 14 days postpartum, a 43 to 48 day reduction in the number of days open was reported. This reduction represented a savings of upto $144 per cow.

However, Holness and Hale (1980) observed that GnRH injection at 30 days after calving was not successful in inducing oestrus. According to Ball and Lamming (1983), failure of induction of oestrus by administration of GnRH was partly due to poor ovarian response to injection and partly to failure in oestrus detection. Etherington *et al.* (1984) reported that GnRH treatment at day 15 postpartum was generally detrimental in cows with uterine infections and found a higher incidence of pyometra following GnRH treatment at day 15 postpartum. Peter *et al.* (1988) reported that intrauterine infections would delay postpartum follicular development and GnRH treatment in such cows was detrimental to resumption of ovarian activity. Stevenson and Call (1988) also reported that cows with reproductive disorders had longer intervals from calving to conception after receiving 100 μg GnRH between day 18 and 25 postpartum.

2.2.2 Prostaglandin F_2 alpha

Lindell *et al.* (1982) found a correlation between duration of uterine involution and PGF_2 alpha concentration. Uterine involution appeared to be most rapid in animals with greater PGF_2 alpha concentration. Prolonged high concentration of PGFM was associated with both faster rate of uterine involution and a stimulatory effect on follicular and luteal components of the ovary (Thatcher *et al.*, 1982). Lindell and Kindahl (1983) reported that administration of 25 mg PGF_2 alpha twice daily from day 3 to day 13 postpartum brought about complete uterine involution by day 13 to 20 after calving. Etherington *et al.* (1984) found that cloprostenol treatment at day 24 postpartum was beneficial to improve the reproductive performance. Treatment with cloprostenol significantly decreased calving to conception and calving to first observed oestrus intervals. Young *et al.* (1984) obtained a first service conception rate of 68 per cent, when a single 25 mg injection of Dinoprost was administered during the period of 14 to 28 days after calving as against 43 per cent in the untreated controls. They further opined that the basal blood progesterone level indicated the absence of an active corpus luteum demonstrating that it was not a luteolytic effect, but a positive myometrial effect. Benmrad and Stevenson (1986) observed that single injection of PGF_2 alpha between 10 and 14 days postpartum reduced intervals to second and third ovulation, shortened the first oestrous cycle and improved fertility. Interval from calving to conception was reduced by 43 to 48 days for cows with an abnormal puerperium treated with PGF_2 alpha compared with controls. Young and Anderson (1986) found that a single injection of dinoprost tromethamine to apparently normal dairy cows between 14 and 28 days after calving significantly improved conception to first service and shortened the interval from calving to conception. Young (1989) found that prostaglandins administered every 12 hours from day 3 to day 13 post calving improved uterine involution. Al-Raheem and Al-Ritha (1990) observed that when

25 mg PGF_2 alpha was given on 24th day of calving, interval to first oestrus averaged 37 ± 6 d, with 83 per cent of cows showing oestrus within 45 d of calving. Wichtel (1991) reported that there was evidence of endogenous prostaglandins in placental detachment and uterine involution during the first three to four weeks after calving. White *et al.* (1996) observed that 25 mg PGF_2 alpha given once 14 to 28 days post partum, reduced number of days open by 43 to 48 days. Research findings indicated that PGF_2 alpha used during the postpartum period would reduce the interval to first oestrus and the number of days open through its effect on uterine involution or ovarian activity before breeding commences.

But Stevenson and Call (1988) observed no profertility effects on administration of 25 mg PGF_2 alpha once between day 11 and 25. Armstrong *et al.* (1989) reported that a single injection of fenprostalene on the day of calving or between days 14 and 21 after calving did not affect the calving to first service interval, the number of services per conception or the conception rate of dairy cows. Archbald *et al.* (1990) observed that PGF_2 alpha treatment of postpartum cows which had experienced dystocia and/or retained fetal membranes had no beneficial effect on fertility during the succeeding postpartum period. Glanvilli and Dobson (1991), Myneuddin *et al.* (1991), Morton *et al.* (1992) and Pinheiro *et al* (1992) found that a single intramuscular injection of 25 mg Dinoprost, between 14 and 28 days after calving had no significant effect on the interval from calving to first service, calving to conception and first service conception rates.

2.3 Assay of serum progesterone

Edqvist *et al.* (1974) observed that progesterone level on day 8 of the oestrous cycle varied between 1.1 and 2.8 ng/ml while on day 14, the levels varied between 3.0 and 7.0 ng/ml. Wishart *et al.* (1975) observed that progesterone levels were low at the beginning of each cycle, increasing from about day 5 to reach levels of 6 ng/ml to 10 ng/ml between day 10 and 16, and abrupt fall to 0.5 ng/ml just prior to the next oestrus. The mean progesterone level on day 20 in pregnant and non-pregnant cows were 7.24 ± 1.42 and 0.76 ± 0.6 ng/ml respectively. They also opined that the accuracy of predicting pregnant and non-pregnant animals by progesterone estimation was 83.5 and 97.0 per cent respectively. Pennington *et al.* (1976) claimed 73 per cent accuracy in predicting pregnancy on the basis of high progesterone levels at day 12, while for predicting non-pregnancy the accuracy was around 95 per cent. Agarwal *et al.* (1977) reported that the average progesterone concentration in non-pregnant cows was around 1.0 ng/ml during the first three days of the cycle, then started increasing and attained a peak value of 2.32 ± 0.44 ng/ml on day 12 which was maintained till day 15; thereafter it declined abruptly and attained a value as low as at the time of oestrus. However, in pregnant cows, a similar trend was found upto day 15 of the cycle, but thereafter the concentration gradually increased till day 21 of the oestrous cycle. They opined that characteristic difference in progesterone concentration between pregnant and non-pregnant cows during the last phase of oestrous cycle might help in diagnosing pregnancy at an early stage of 19 to 20 days. *Perera et al.* (1980) opined that the determination of plasma progesterone concentration 21 days after insemination was an accurate method of predicting non-pregnancy in buffaloes (97.1 per cent). Singh and Puthiyandy (1980) observed a peak mean progesterone concentration 4.97 ± 1.97 ng/ml on day 15 of cycle which declined to basal value of 0.20 ± 0.07 ng/ml on the day of subsequent oestrus in non-pregnant buffaloes. Chauhan and Sharma (1983) reported that the accuracy of predicting pregnancy and non-pregnancy in buffaloes based on the serum progesterone level on day 21 was 71.4 and 100 per cent respectively. Roche *et al.* (1985) observed that blood concentrations of progesterone was not different before insemination and for 16 days after insemination for pregnant and non-pregnant heifers. However, between days 17 and 20, progesterone concentrations declined in non-pregnant heifers. Davies and Fletcher (1987) remarked that serum separation should be carried out as soon as possible for the qualitative assessment of progesterone to be accurate by enzyme immunoassay. Robinson *et al.* (1989) reported that the mean plasma progesterone concentration remained elevated after day 16 in pregnant cows while it declined in those which are not pregnant.

Materials and Methods

MATERIALS AND METHODS

The material for the study consisted of 30 crossbred cows belonging to Livestock Research Station, Thiruvazhamkunnu of Kerala Agricultural University, maintained under identical conditions of feeding and management. These cows which had normal parturition were allotted randomly into the following treatment groups.

Group I

Ten animals were administered *synthetic gonadotrophin releasing hormone analogue (GnRH analogue, 20 μg) intramuscularly once on the 14th day of calving.

Group II

Ten animals were administered **prostaglandin F_2 alpha (PGF_2 alpha, 25 mg) intramuscularly once on the 14th day of calving.

Group III

Ten animals were administered normal saline, 5 ml intramuscularly once on 14th day of calving.

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* Receptal (Inj.) 10 ml (Hoechst). Each ml contains Buserelin acetate 0.0042 mg equivalent to 0.004 mg Buserelin, 10 mg Benzyl alcohol.

** Dinofertin (Inj.) 5 ml (Alved). Each ml contains Dinoprost, 5 mg

All the animals in each group were examined per rectum for assessing ovarian status and uterine involution. After commencement of the treatment, both experimental and control animals, were kept under close observation for manifestation of oestrus sign and subjected to periodical clinico-gynaecological examination for confirmation of ovulation. Serum progesterone level was estimated 10 days after the first exhibited oestrus by ELISA technique. The animals in all the groups were inseminated in the first oestrus after 45 days of calving with good quality frozen semen. Serum progesterone levels were estimated on day ten and twenty after insemination. Those animals which failed to settle with first insemination were reinseminated on subsequent oestrus. Pregnancy diagnosis was done by rectal examination 45 days after insemination.

The following observations were made:

3.1 Ovarian and uterine status

Regression of pregnancy corpus luteum was assessed by daily rectal examination starting from day 7 postpartum until the corpus luteum was no more palpable. Involution of uterus was considered complete when the uterus regained its nearly normal pregravid location, size and tone.

3.2 Oestrus response

The number and percentage of the animals which responded to the treatment by exhibition of oestrus was recorded.

3.3 Time interval from treatment to first oestrus

Each animal after treatment on day 14 postpartum was closely observed for behavioural signs of heat and those found in oestrus were confirmed by rectal examination of the genital tract. The interval from the treatment to the onset of first oestrus was recorded.

3.4 Time interval from calving to first oestrus

3.5 Intensity of oestrus

The intensity of oestrus was graded as high, medium and low from clinical and behavioural manifestations (Sharma *et al.*, 1968).

3.6 Time interval from calving to first insemination

3.7 Time interval from calving to conception

The interval from calving to the effective service was recorded.

3.8 Conception rate

The first service conception rate and overall conception rate in each group was calculated.

3.9 Estimation of serum progesterone by ELISA technique

Blood collections were made on day 10 after first exhibited oestrus within 45 d of calving and on days 10 and 20 after insemination. Serum was separated by centrifugation and stored at -20°C till processed for progesterone assay. The progesterone concentration was determined by ELISA/competition using streptavidin technology (ENZYMUN-TEST Progesterone by Boehringer Mannheim immuno diagnostics). The sensitivity of the assay ranged from 0-30 ng/ml. The pregnancies predicted on the basis of serum progesterone levels on day 10 and 20 after insemination was confirmed by rectal palpation 45 d after insemination.

The data were subjected to statistical analysis as per Snedecor and Cochran (1967).

Results

RESULTS

Results of investigation on the fertility management of cows during early post partum period using GnRH analogue and Prostaglandin F_2 alpha are presented in Tables 1 to 15 and Fig.1 to 13.

4.1 Involution of genitalia

4.1.1 Regression of pregnancy corpus luteum

Table 1 presents the time taken for regression of pregnancy corpus luteum. It could be noted that by day 15, ovaries developed soft and smooth consistency in all animals indicating the development of follicles. It could be noted that the time taken for regression of pregnancy corpus luteum was not varied between experimental and control animals. Parity did not influence the time taken for the regression of pregnancy corpus luteum.

4.1.2 Involution of uterus

Time taken for the involution of uterus is presented in Table 2 and Fig.1. In group I, II and III, involution was complete in 25.3 ± 0.47 d (23-27 d), 25 ± 0.77 d (22-29 d) and 34.6 ± 1.79 d (28-46 d) respectively. Significant variation was observed between treatment and control groups. However, the time taken for uterine involution between the two treatment groups was not significantly different. Pluriparous cows took apparently longer period for uterine involution than primiparous cows in all three groups (Table 3).

4.2 Interval from calving to first exhibited oestrus

Time taken for the first exhibited oestrus after calving in animals belonging to the three groups is presented in Table 4 and Fig.2. In group I, II and III, the time interval from calving to first exhibited oestrus ranged from 27 to 44 d (32.1 ± 1.69 d), 26 to 48 d (33.5 ± 1.93 d) and 34 to 64 d (46.11 ± 3.19 d) respectively. Time interval from calving to first exhibited oestrus between treatment and control groups was statistically different.

It could also be noticed that in group I, all the animals (100%) exhibited oestrus within 45 d of calving, while in group II, 90 per cent (9 out of 10) exhibited oestrus within 45 d post partum. However, in the control group, only 50 per cent (5 out of 10) exhibited oestrus within 45 d of calving (Table 5 and Fig.3). Statistical analysis of data revealed that this variation between treatment and control groups was significantly different. However, between treatment groups there was no significant difference.

4.3 Interval from treatment to onset of oestrus

Time taken from treatment to onset of oestrus in group I, II and III ranged from 13 to 24 d (18.1 ± 1.69 d), 12 to 34 d (19.5 ± 1.93 d) and 20 to 50 d (30.7 ± 3.37 d) respectively (Table 6 and Fig.4). It was found that experimental animals evinced oestrus significantly earlier than those in the control group. However, there was no significant difference between the treatment groups.

4.4 Intensity of oestrus

Intensity of oestrus among experimental and control animals is presented in Table 7 and Fig.5. Percentage of cows that exhibited high, medium and low intensity of oestrus was 20,50 and 30 respectively in group I; 10, 40 and 50 respectively in group II. In the control group, 33.33 and 66.66 per cent of animals exhibited medium and low intensity of oestrus respectively while none exhibited high intensity of oestrus. Statistical analysis revealed no significant difference between treatment and control groups with respect to intensity of oestrus. But there was a proportionately higher number of cows exhibiting high and medium intensity of oestrus in both the treatment groups when compared to those in control. Data furnished in Table 3 revealed high and medium intensity of oestrus proportionately higher in cows with first parity in all the three groups.

4.5 Serum progesterone level 10 d after exhibited oestrus

The rate of ovulation in animals that exhibited oestrus upto 45 d of calving was 90 per cent in group I, 77.77 per cent in group II and 60 per cent in group III. However, this variation was not significantly different (Table 9).

Serum progesterone concentration 10 d after first exhibited oestrus within 45 d of calving was determined by ELISA. In group I, progesterone level ranged from 3.2 to 6.3 ng/ml with a mean of 4.76 ± 0.33 ng/ml for animals that ovulated and 2.0 ng/ml for the anovulated. In group II and III, the concentration ranged from 4.1 to 6.2 (5.12 ± 0.31) and 2.7 to 3.0 ng/ml (2.86 ± 0.09) for the ovulated while for the anovulated it ranged from 1.5 to 2.5 (2.0) and 1 to 1.5 (1.25) ng/ml respectively (Table 10 and Fig.6). Statistical analysis of data revealed this variation between treatment and control groups as significant. However, between group I and II, there was no significant difference.

4.6 Interval from calving to first insemination

The interval from calving to first insemination in group I, II and III ranged from 48 to 62 d (56 ± 1.99 d), 47 to 61 d (52 ± 1.24 d) and 54 to 84 d (65.77 ± 2.90 d) respectively (Table 11 and Fig.7). Statistical analysis revealed significant difference between treatment and control groups. However, there was no significant difference between group I and II with respect to interval from calving to first insemination. Parity did not influence the interval from calving to first insemination.

4.7 Conception rate

Conception rates in the experimental and control group are presented in Table 12 and Fig.8. The first insemination conception rate, second insemination conception rate and overall conception rate in group I were 30, 50 and 90 per cent respectively as against corresponding values of 20, 30 and 70 per cent in group II and 11.1, 11.1 and 55.55 per cent in group III. There was no significant difference in first insemination conception rate between the treatment and control groups. However, there was significant difference in the overall conception rate between group I and III, while an apparently higher overall conception rate was obtained in group II when compared to that of group III.

The average number of inseminations required per conception for the three groups is furnished in Table 12 and Fig.9. The average number of inseminations required per conception was 1.7, 2.25 and 2.8 respectively for group I, II and III. However, this variation was not significantly different, eventhough the AI index was much lower in group I than in group II and III.

4.8 Interval from calving to conception

Interval from calving to conception for group I, II and III ranged from 61 to 95 d (69.77 ± 3.70), 61 to 103 d (75.8 ± 5.62 d) and 66 to 108 d (95 ± 6.04 d) respectively (Table 13 and Fig.10). Statistical analysis of data revealed significant difference between treatment and control groups. However, the difference was not significant between group I and II.

4.9 Early pregnancy diagnosis from serum progesterone concentration

Serum progesterone level 10 and 20 days after insemination was determined by ELISA technique. The pregnant cows, in general, had higher concentration, on day 10 and 20 after insemination. The main difference from non-pregnant cows was that the concentration did not fall from the value on day 10, rather it showed a rising trend to day 20 (Fig.11 to 13). Twenty animals were predicted pregnant on the basis of higher progesterone concentration on day 20 (mean 7.06 ± 0.25 ng/ml) than on day 10 (mean 5.985 ± 0.22 ng/ml) after insemination. Out of this, fourteen cows were confirmed pregnant (70%) by rectal palpation 45 d after insemination. seventeen animals were predicted non-pregnant on the basis of low progesterone concentration on day 20 (mean 1.576 ± 0.14 ng/ml) than on day 10 (mean 5.806 ± 0.24 ng/ml) after insemination. All these were confirmed non-pregnant (100%) by rectal palpation on day 45 after insemination (Tables 14 and 15).

Table 1. Time taken for regression of pregnancy C.L.

Group	Number of animals	Range (days)	Mean
I	10	13-16	14.5 ± 0.37
II	10	13-17	14.9 ± 0.45
III	10	12-22	15.3 ± 0.87

Inference: Time taken for regression of pregnancy corpus luteum between treatment and control groups was not significantly different

Table 2. Effect of treatments on uterine involution

Group	Number of animals	Range (days)	Mean (days)
I	10	23-27	25.3 ± 0.47
II	10	22-29	25.0 ± 0.77
III	10	28-46	34.6 ± 1.79

Inference: Time taken for uterine involution between treatment and control groups was significantly different ($P<0.01$). Between Group I and II, there was no significant difference

Table 3. Effect of parity on uterine involution

	Group I	Group II	Group III
Primipara	24.6	22.25	31.5
Pleuripara	26	26.5	36.6

Table 4. Time interval from calving to first oestrus

Group	Number of animals	Interval from calving to first oestrus (days)	
		Range	Mean
I	10	27-44	32.1 ± 1.69
II	10	26-48	33.5 ± 1.93
III	9	34-64	46.11± 3.19

Inference: Time interval from calving to first oestrus between treatment and control groups was significantly different ($P<0.01$). Between Group I and II, there was no significant difference

Table 5. Percentage of cows that evinced oestrus within 45 d of calving

Group	Number of animals treated	Number of animals that evinced oestrus	Percentage
I	10	10	100
II	10	9	90
III	10	5	50

Inference: Oestrus response within 45 days of calving between treatment and control groups was significantly different (P<0.01). Between Group I and II, there was no significant difference

Table 6. Time taken from treatment to onset of oestrus

Groups	Number of animals	Time interval from treatment to first exhibited oestrus	
		Range (days)	Mean
I	10	13-24	18.1 ± 1.69
II	10	12-34	19.5 ± 1.93
III	9	20-50	30.7 ± 3.37

Inference: Time taken from treatment to onset of oestrus between treatment and control groups was significantly different (P<0.01). Between Group I and II, there was no significant difference

Table 7. Intensity of oestrus

Group	Number of animals	Intensity of oestrus					
		High		Medium		Low	
		No.	%	No.	%	No.	%
I	10	2	20	5	50	3	30
II	10	1	10	4	40	5	50
III	9	0	0	3	33.33	6	66.66

Inference: There was no significant difference between treatment and control groups with respect to high, medium and low intensity of oestrus

Table 8. Effect of parity on intensity of oestrus

	Group I			Group II			Group III		
	H	M	L	H	M	L	H	M	L
Primipara	2	3	-	1	2	1	-	2	2
Pleuripara	-	2	3	-	2	4	-	1	4

Table 9. Ovulation rate at oestrus within 45 d of calving

	Group I	Group II	Group III
Number of animals	10	9	5
Number ovulated	9	7	3
Percentage	90	77.77	60

Inference: No significant difference was noticed between treatment and control groups with respect to ovulation rate at oestrus within 45 d of calving

Table 10. Serum progesterone concentration 10 d after exhibited oestrus

Groups	Ovulated (ng/ml)		Anovulated (ng/ml)	
	Range	Mean	Range	Mean
I	3.2-6.3	4.76 ±0.33	-	2.0
II	4.1-6.2	5.128±0.31	1.5-2.5	2.0
III	2.7-3.0	2.86 ±0.09	1-1.5	1.25

Table 11. Interval from calving to first insemination

Groups	Days to first insemination	
	Range	Mean
I	48-62	56.00 ± 1.99
II	47-61	52.00 ± 1.24
III	54-84	65.77 ± 2.90

Inference: Interval from calving to first insemination between treatment and control groups was significantly different (P<0.01). Between Group I and II, there was no significant difference

Table 12. Conception rate and number of inseminations per conception

Groups	Number of animals	First insemination conception		Second insemination conception		Three & above insemination per conception		Average number of insemination per conception	Overall conception rate*	
		No.	%	No.	%	No.	%		No.	%
I	10	3	30	5	50	1	10	1.7	9	90
II	10	2	20	3	30	3	30	2.25	7	70
III	9	1	11.1	1	11.1	5	55.55	2.8	5	55.55

Inference: First insemination conception rate between treatment and control groups was not significantly different. Second insemination conception rate between Group I and III was significantly different (P<0.01). Overall conception rate between Group I and group III was significantly different. However, between Group I and Group II, there was no significant difference

Table 13. Interval from calving to conception

Groups	Days to conception	
	Range	Mean
I	61-95	69.77 ± 3.70
II	61-103	75.87 ± 5.62
III	66-108	95.00 ± 6.04

Inference: Interval from calving to conception between treatment and control groups was significantly different (P<0.01). Between Group I and II, there was no significant difference

Table 14. Serum progesterone concentration on days 10 and 20 after insemination

Category	Serum progesterone (ng/ml)			
	Day 10		Day 20	
	Mean	Range	Mean	Range
Predicted pregnant	5.985±0.22	4.1-7.5	7.060±0.25	5.0-8.7
Predicted non-pregnant	5.806±0.24	4.3-7.6	1.576±0.14	0.7-2.6

Table 15. Accuracy of predicting pregnancy and non-pregnancy from serum progesterone level on days 10 and 20

Category	Predicted	Confirmed	Accuracy (%)
Pregnant	20	14	70
Non-pregnant	17	17	100

Fig. 1

TIME TAKEN FOR UTERINE INVOLUTION

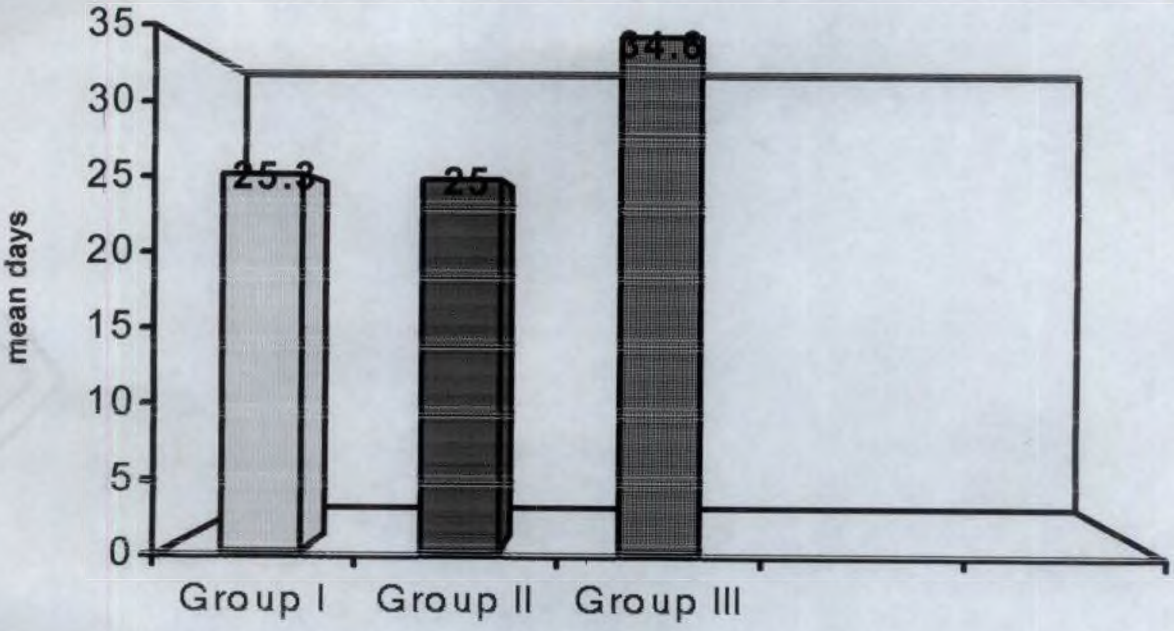


Fig. 2

TIME INTERVAL FROM CALVING TO FIRST OESTRUS

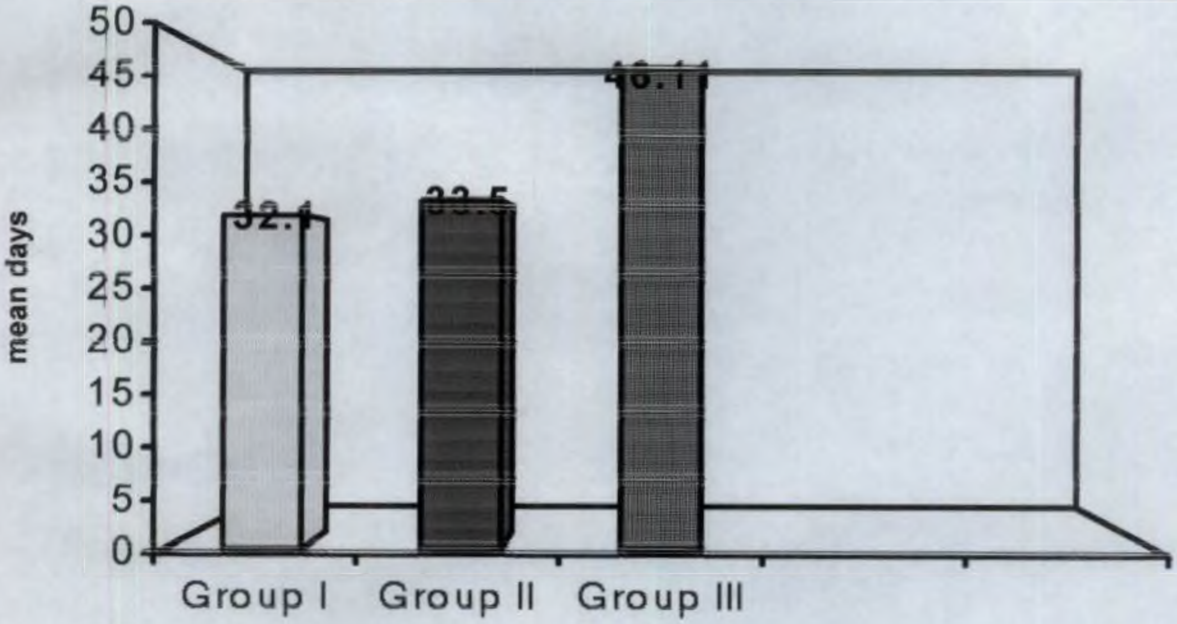


Fig. 3

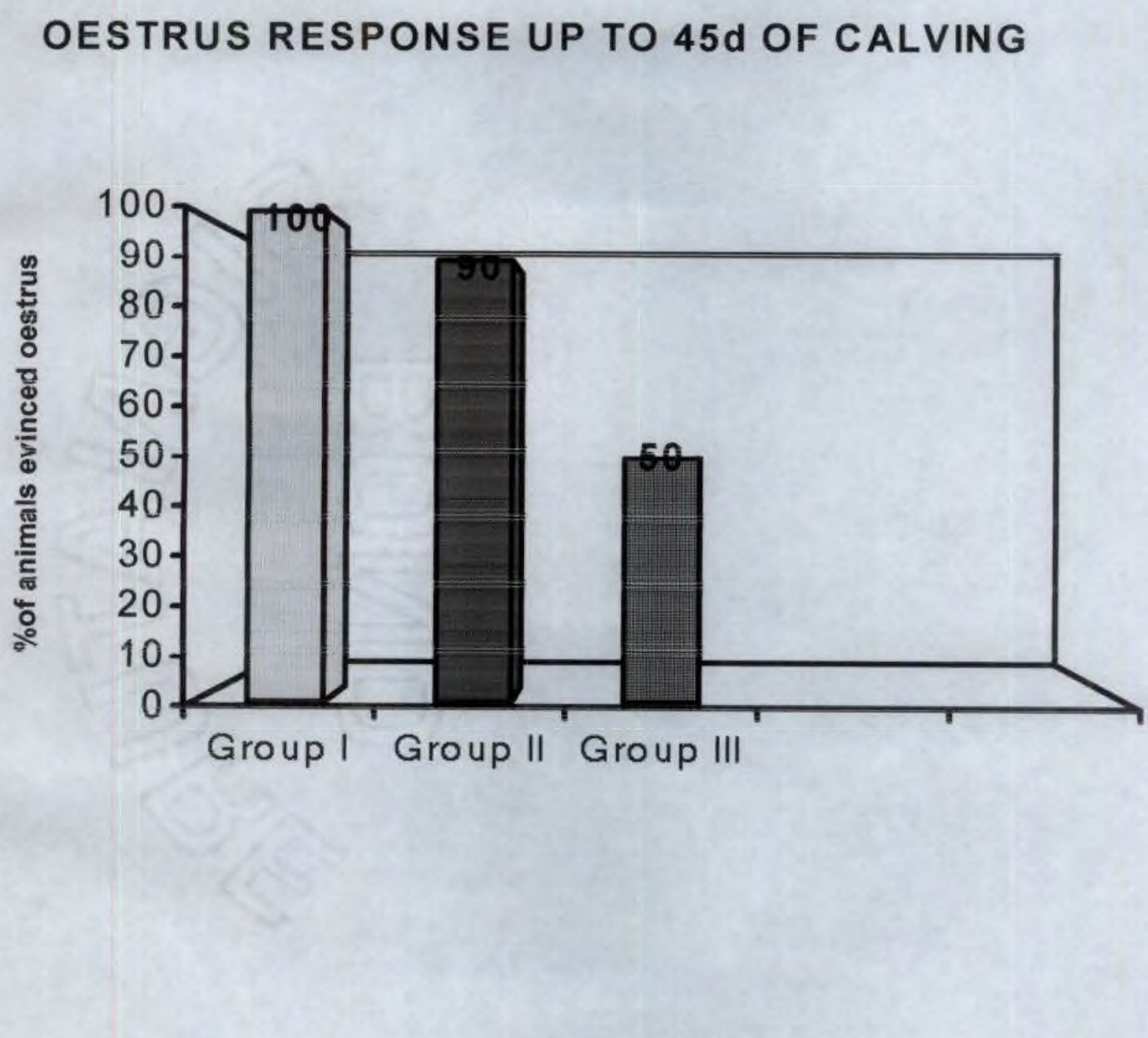

OESTRUS RESPONSE UP TO 45d OF CALVING
%of animals evinced oestrus
100
90
80
70
60
50
40
30
20
10
0
100
90
50
Group I
Group II
Group III

Fig. 4

TIME INTERVAL FROM TREATMENT TO FIRST OESTRUS

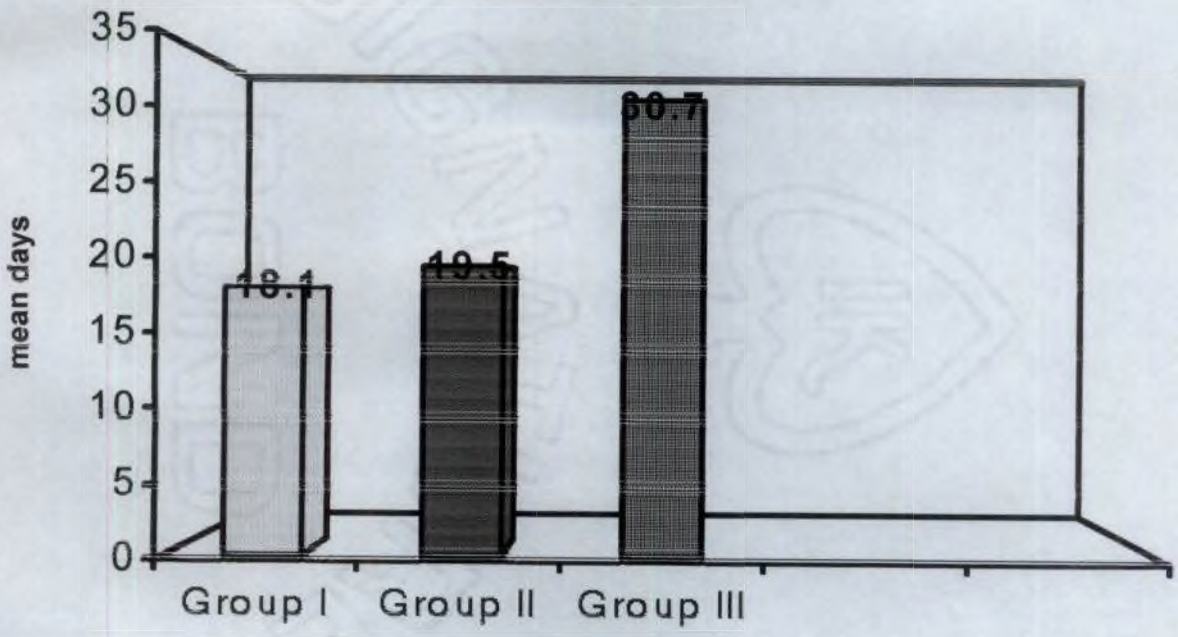


Fig. 5

INTENSITY OF OESTRUS

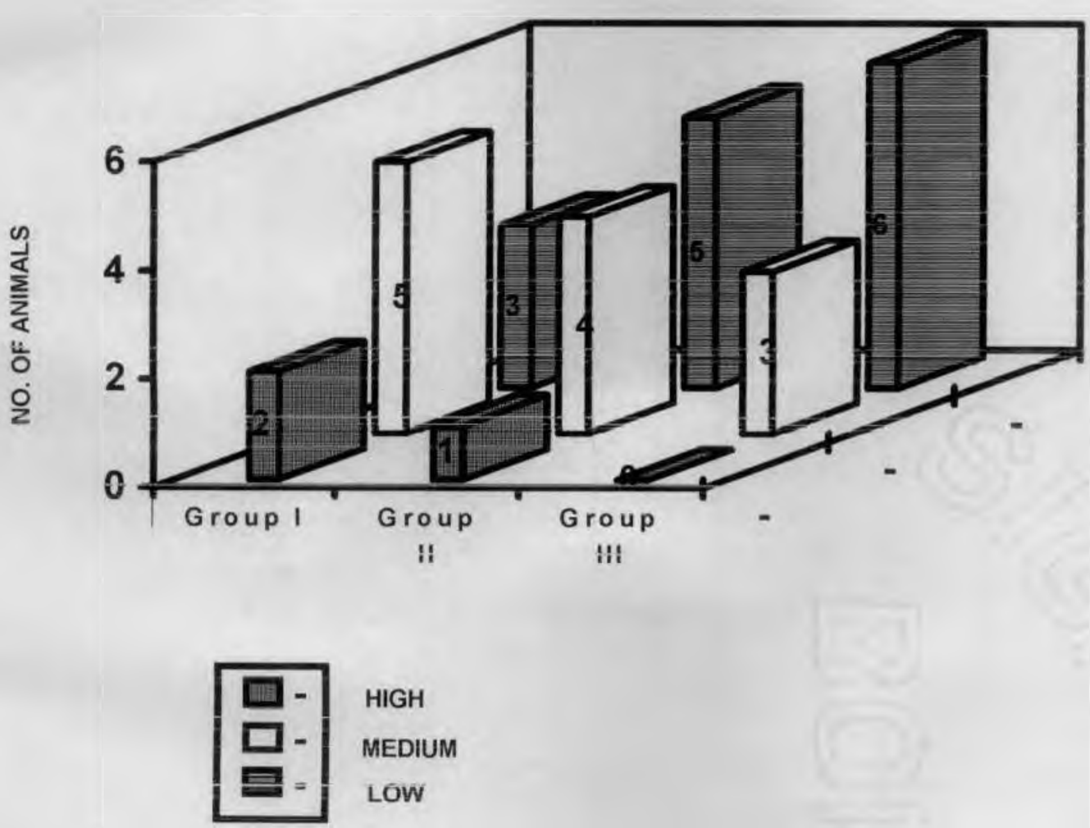


Fig. 6

SERUM PROGESTERONE LEVEL 10d AFTER EXHIBITED OESTRUS

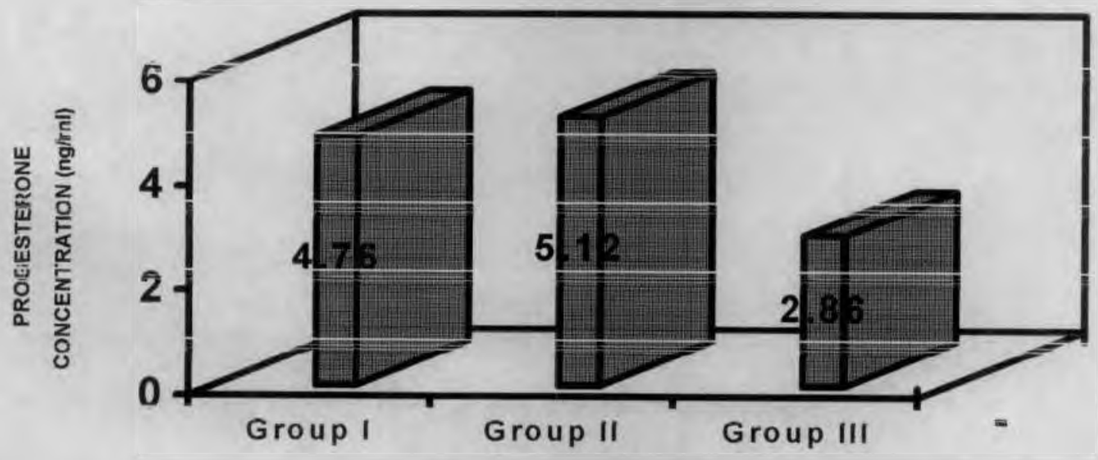


Fig. 7

INTERVAL FROM CALVING TO FIRST INSEMINATION

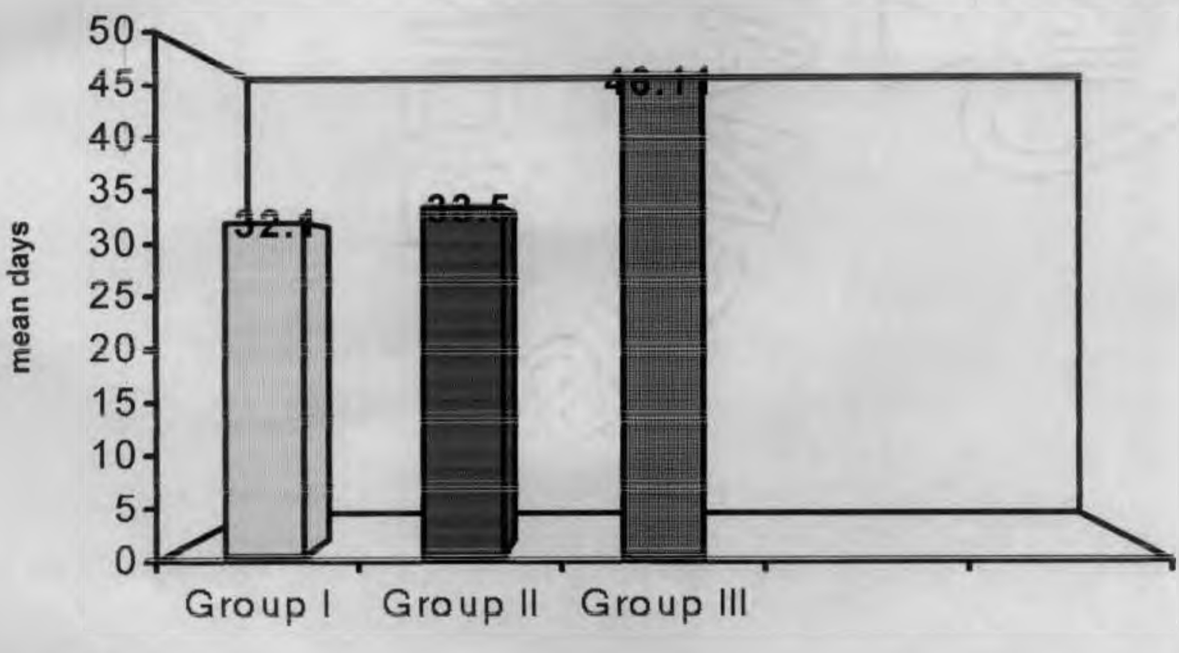


Fig. 8

CONCEPTION RATES AND OVERALL CONCEPTION RATES

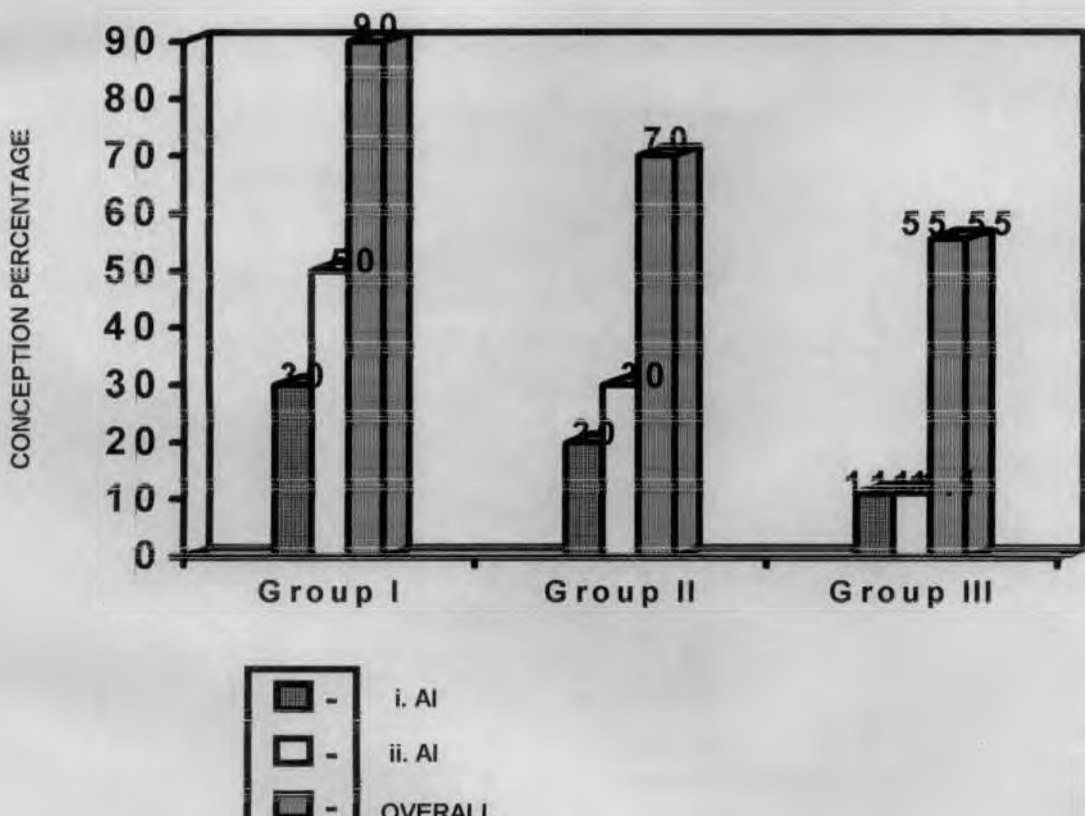


Fig. 9

AVERAGE NUMBER OF INSEMINATIONS PER CONCEPTION

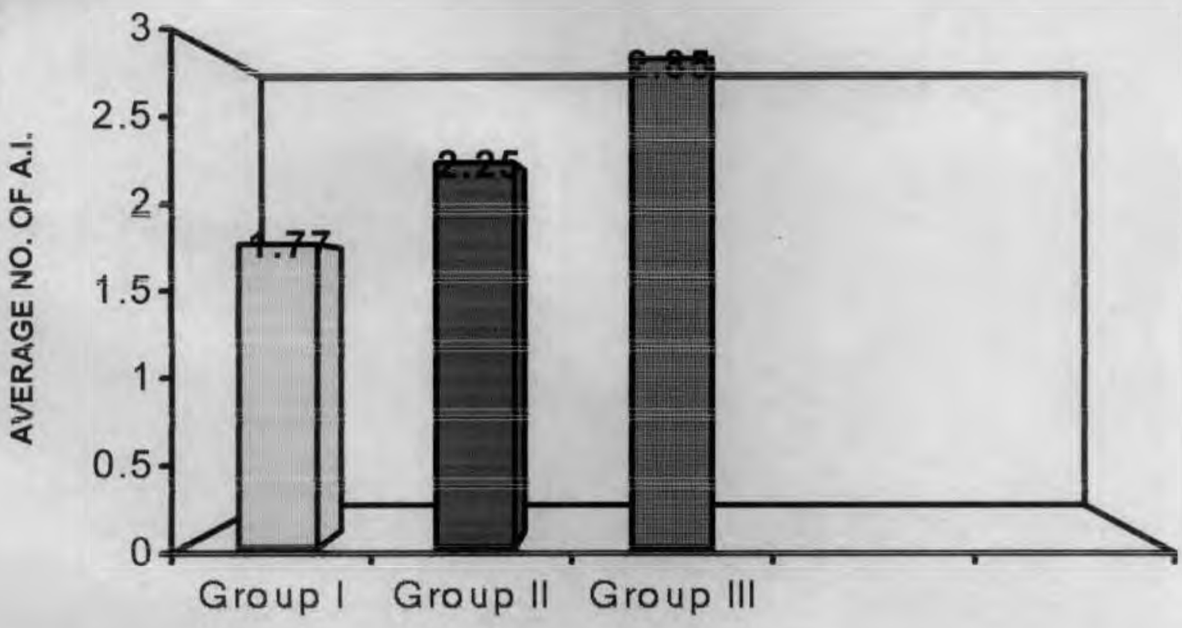


Fig. 10

INTERVAL FROM CALVING TO CONCEPTION

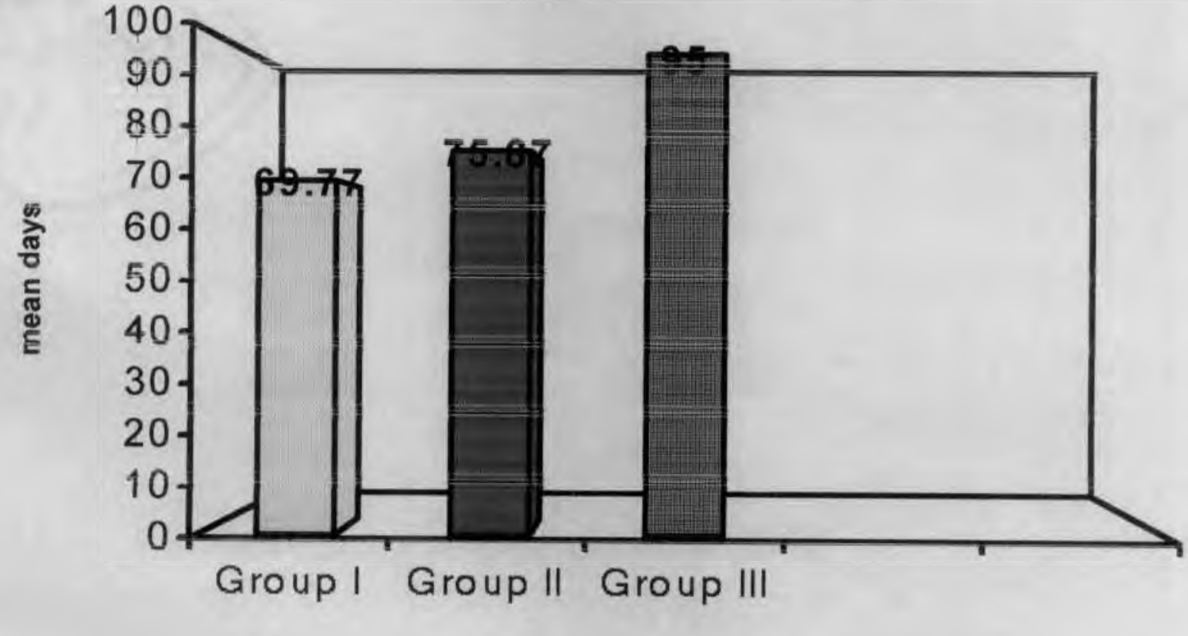


Fig. 11

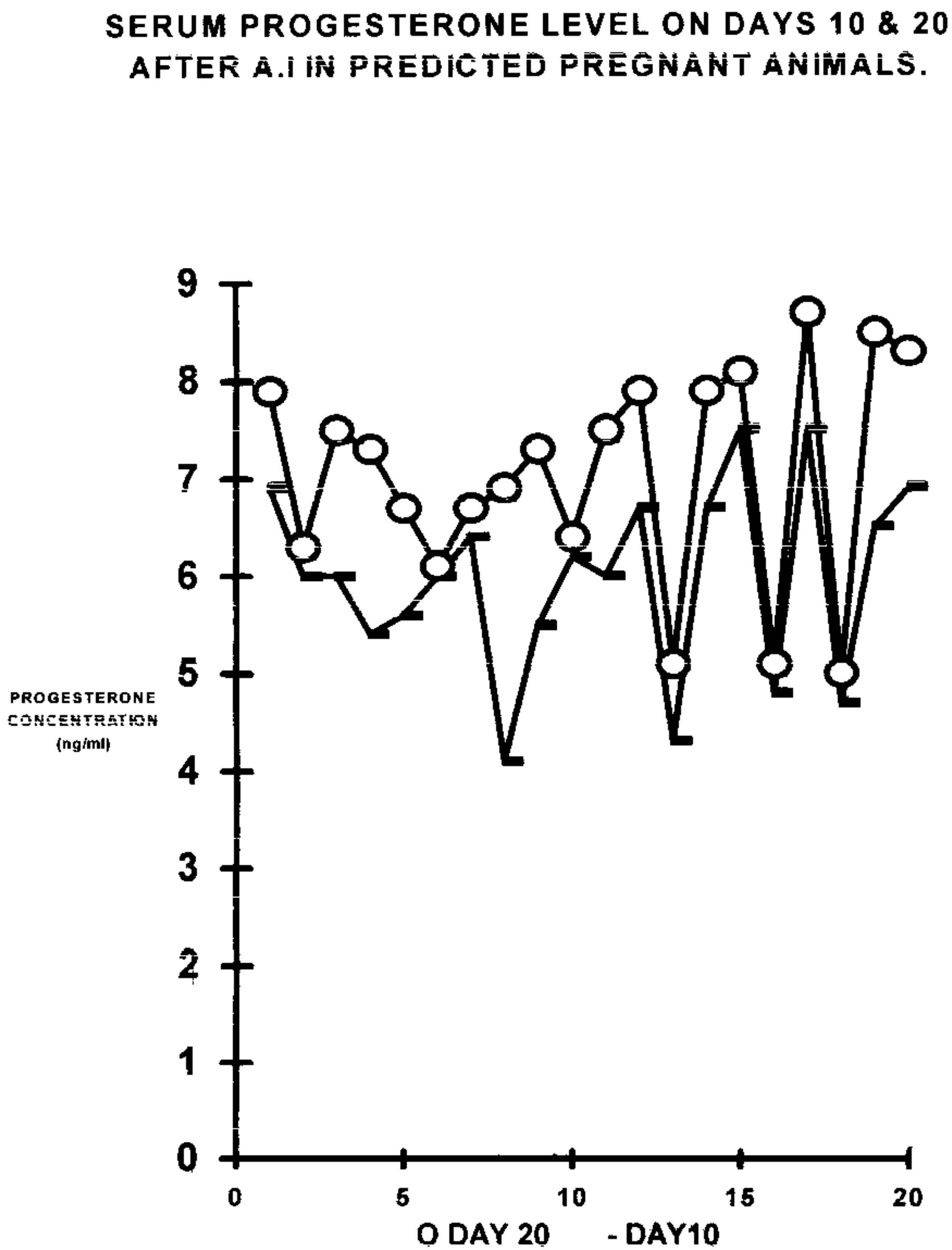

SERUM PROGESTERONE LEVEL ON DAYS 10 & 20
AFTER A.I IN PREDICTED PREGNANT ANIMALS.
PROGESTERONE
CONCENTRATION
(ng/ml)
9
8
7
6
5
4
3
2
1
0
0
5
10
15
20
O DAY 20
- DAY10

Fig. 12

SERUM PROGESTERONE LEVEL ON DAYS 10 & 20 AFTER A.I
IN PREDICTED NON-PREGNANT ANIMALS.

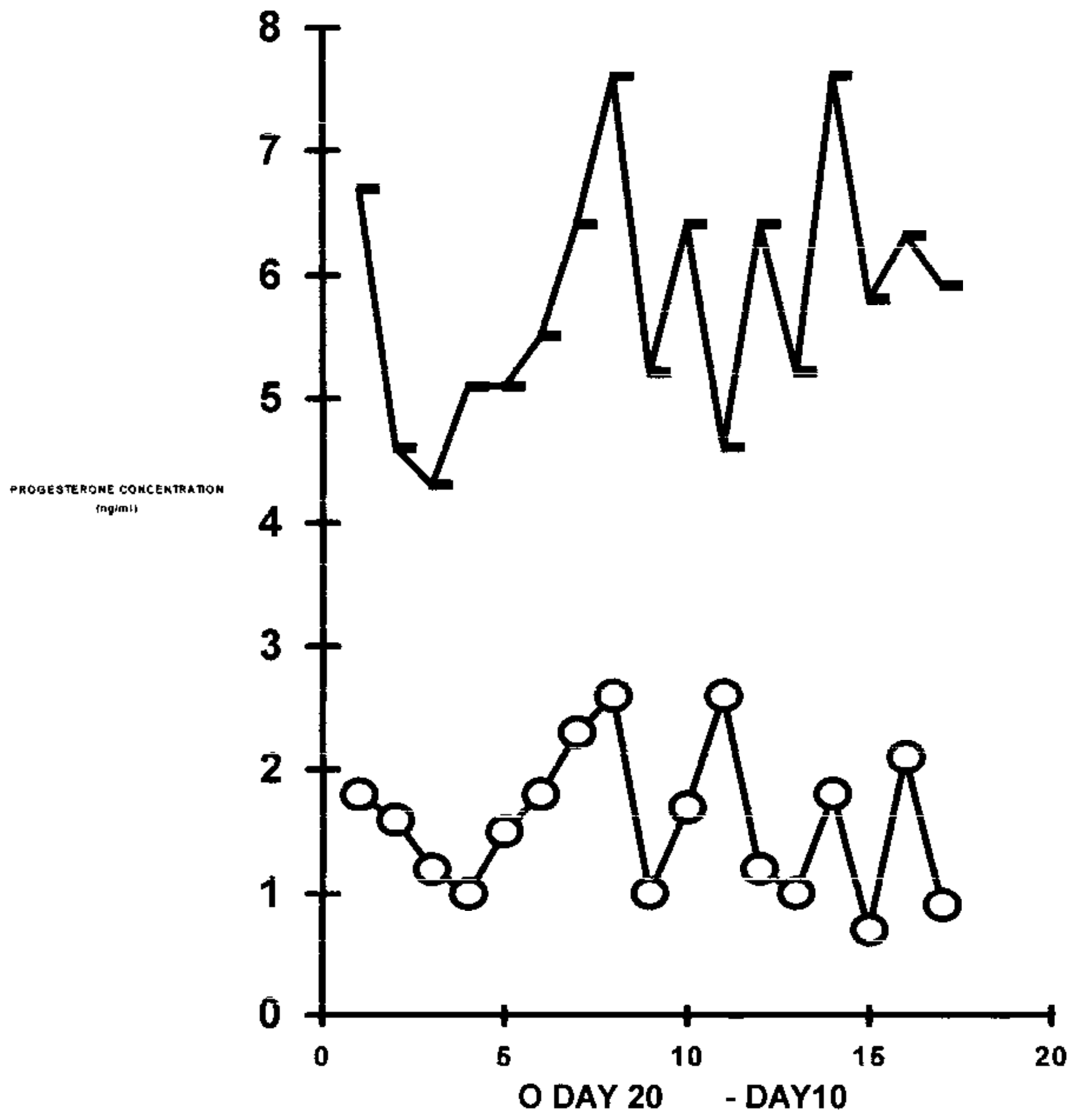
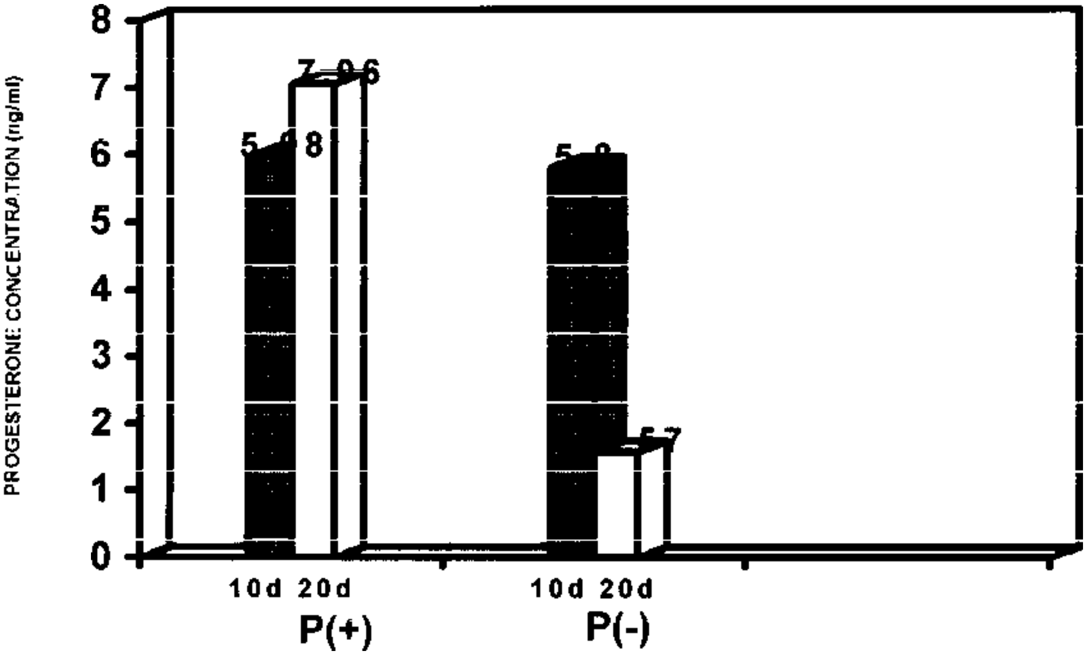


Fig. 13

SERUM PROGESTERONE LEVEL ON DAY 10 & 20 AFTER A.I



Discussion

DISCUSSION

The calving interval is perhaps the major parameter used to assess bovine reproductive efficiency which determines the dairy productivity in terms of total milk and calf productivity. A prolonged intercalving period results in spending a greater proportion of lactation in the less profitable part of lactation curve. Post partum oestrus and interval to first service are the most important factors affecting calving interval since they are highly correlated. Delayed uterine involution is associated with delayed post partum oestrus which is one of the factors responsible for an undesirable extension of calving to conception interval. The primary defect that exists in post partum cow is a low serum concentration of LH caused by low frequency pulsatile secretion pattern of LH and presumably GnRH. Administration of exogenous hormones to induce early post partum oestrus in cows has given varying results.

An acceptable approach should, however, include treatments that accelerate uterine involution and hasten fertile ovarian cyclicity. Gonadotrophin releasing hormone because of its ability to release FSH and LH from pituitary has been the drug of choice for early induction of post partum oestrus. With the chemical identification and synthesis of GnRH, a new and powerful drug has been in use for induction of early post partum oestrus in cattle. Further, alterations in the chemical structure of native GnRH molecule have led to the synthesis of potent GnRH analogue. But owing to the alteration in chemical structure, marked differences exist between various GnRH analogues in releasing LH and FSH in cattle (Nawito *et al.*, 1977; Chenault *et al.*, 1990). Buserelin which is the ingredient of "Receptal" is 50 times more potent than gonadorelin. Beneficial effects of "Receptal" in early post partum cows have been reported by Nash *et al.* (1980), Benmrad and Stevenson (1986), Pattabiraman (1986) and White *et al.* (1996).

Prostaglandin F_2 alpha, a derivative of the unsaturated hydroxy fatty acids, linolenic and arachidonic acids causes regression of corpus luteum and has stimulating effect on smooth muscles. PGF_2 alpha has been used in clinical situations where regression of the corpus luteum or stimulation of smooth muscles was desired. "Dinofertin" is the natural PGF_2 alpha (Dinoprost) which has no side effects usually associated with synthetic analogues. Beneficial effects of Dinoprost in early post partum cows have been reported by Young *et al.* (1984), Young and Anderson (1986) and White *et al.* (1996).

The present investigation was, therefore, taken up with the object of studying the efficacy of administration of

"Receptal" a GnRH analogue and "Dinofertin" natural PGF_2 alpha in inducing early post partum oestrus thereby reducing the calving to conception interval.

The material used for the present study consisted of 30 crossbred cows belonging to Livestock Research Station, Thiruvazhamkunnu maintained under identical conditions of feeding and management. These cows which had normal parturition were allotted randomly into three different treatment groups. Ten animals in group I were administered intramuscularly "Receptal" 5 ml once on 14th day of calving. Ten animals in Group II, were administered intramuscularly "Dinofertin" 5 ml once on 14th day of calving. Ten cows in Group III were administered 5 ml normal saline intramuscularly once on 14th day of calving and formed the control.

5.1 Involution of genitalia

Time taken for the involution of uterus is presented in Table 2 and Fig.1. In the present study, animals in Group I and II completed uterine involution by 25.3 ± 0.47 and 25 ± 0.77 days respectively while animals in control group seemed to have completed uterine involution by 34.6 ± 1.79 days. Statistical analysis revealed significant difference in time taken for uterine involution between animals of experimental and control groups. The present study thus indicates that

`Receptal' when administered on day 14 after calving hastened the rate of uterine involution. This is in agreement with reports by Britt (1974), Zaied *et al.* (1980), Cavestany and Foote (1985), Benmrad and Stevenson (1986), Aboul-Ela and El-Keraby (1986), Bagal and Kadu (1990) and Barkawi and Farghacy (1995). Britt (1974) opined that beneficial effect of GnRH in hastening rate of uterine involution might be possibly due to early resumption of ovarian function induced by LH response to treatment. However, in control animals, that had not ovulated prior to day 20, uterine size would be expected to be larger due to water imbibition effect of oestrogen. Further, Benmrad and Stevenson (1986) opined that the effect of GnRH in uterine involution, remains unelucidated. The comparatively good response to "Receptal" in uterine involution might therefore be attributed to the early resumption of ovarian function.

Treatment of animals with 'Dinofertin' on day 14 after calving (Group II) influenced the rate of uterine involution as reported by Lindell *et al.* (1982), Lindell and Kindahl (1983), Young *et al.* (1984), Benmrad and Stevenson (1986), Young and Anderson (1986), Armstrong *et al.* (1989), Young (1989), Saturnino *et al.* (1992) and White *et al.* (1996). Prostaglandin F_2 alpha has been associated with rapid uterine involution (Eley *et al.*, 1979, Lindell *et al.*, 1982, Madej *et al.*, 1984). Beneficial effect of prostaglandin F_2 alpha in uterine involution was attributed to the smooth muscle contracting activity on myometrium (Young *et al.*, 1984, Benmrad and Stevenson, 1986, Young and Anderson, 1986, Young, 1989). Oltenacu *et al.* (1983) found tardy uterine involution due to intrauterine infection. Lindell *et al.* (1982) and White *et al.* (1996) observed that prostaglandin administration improved uterine tone that reduced the uterine infections and hastened uterine involution. The faster rate of uterine involution in group II animals treated with 'Dinofertin' might therefore be attributed to the smooth muscle contractile potential of prostaglandin.

5.2 Interval from calving to first exhibited oestrus

Interval from calving to first exhibited oestrus in animals belonging to the three groups is presented in Table 4 and Fig.2. It could be seen that among 'Receptal' treated animals, the interval from calving to oestrus ranged from 27 to 44 d with a mean of 32.1 ± 1.69 d while in 'Dinofertin' treated cows, the interval ranged from 26 to 48 d with a mean of 33.5 ± 1.93 d. However, the control animals took 46.1 ± 3.19 d on an average with a range of 34 to 64 d for interval from calving to first oestrus. Statistical analysis of data revealed significant difference in this respect between experimental and control animals. This is in accordance with the findings of earlier workers like Aboul-Ela and El-Keraby

(1986), Benmrad and Stevenson (1986), Cavestany and Foote (1986), Pattabiraman *et al.* (1986), Al-Raheem and Al-Ritha (1990), Archbald (1990). The shorter interval from calving to onset of first exhibited oestrus could be attributed to the release of LH in cows during early postpartum period facilitated by GnRH (Britt *et al.*, 1977; Kesler *et al.*, 1977; Peters and Lamming, 1984; Roche *et al.*, 1992). However, conflicting views were expressed regarding the time interval from calving to first oestrus. Britt (1974) reported a longer period for the exhibition of oestrus after calving eventhough GnRH treatment resulted in a shorter interval to first ovulation. Stevenson and Call (1988) reported that the average time interval from calving to first oestrus was 61 d after administration of GnRH. Lee and Kim (1994) observed that GnRH treatment on day 14 brought the animals into oestrus at an average of 51.7 d after calving. It might be due to the fact that follicular growth and maturation are important to GnRH induced initiation of cyclic ovarian activity (Kesler *et al.*, 1977). These variation might partly be due to variations in ovarian response at the time of administration of the drug (Ball and Lamming, 1983). It may also be attributed to the variation in the time of administration of 'Receptal' in the postpartum period and agreed favourably with the response of Zaied *et al.* (1980), Brown (1985) and Pattabiraman *et al.* (1986).

The results obtained with respect to interval from calving to first oestrus in 'Dinofertin' treated animals finds agreement with that of reports by Lindell and Kindahl (1983), Etherington *et al.* (1984), Young *et al.* (1984), Benmrad and Stevenson (1986), Young and Anderson (1986), Etherington *et al.* (1988), Al-Raheem and Al-Ritha (1990) and White *et al.* (1996). The shorter interval to first exhibited oestrus after calving in prostaglandin F_2 alpha treated animals could be attributed to the rapid uterine involution due to smooth muscle contracting activity on myometrium. On the contrary, Macmillan *et al.* (1987), Armstrong *et al.* (1989) and Morton *et al.* (1992) observed no significant effect. This may be attributed to the variation in period after calving at which treatment was given. The treatment was carried out at a longer interval after calving.

5.3 Interval from treatment to onset of oestrus

Perusal of data in Table 6 and Fig.4 revealed that in group I and II, the interval from treatment to onset of oestrus ranged from 13 to 24 d (18.1 ± 1.69 d) and 12 to 34 d (19.5 ± 1.93 d) respectively. However, the control animals in group III took 20 to 50 d (30.7 ± 3.37 d) for onset of oestrus. The interval from treatment to onset of oestrus between experimental and control animals was statistically significant. The result obtained with GnRH is in agreement with the earlier reports by Benmrad and Stevenson (1986) and Pattabiraman *et al.* (1986). The shorter interval from GnRH treatment to onset of first exhibited oestrus could be attributed to the early resumption of ovarian activity induced by increased LH pulse frequency in response to GnRH. However, Bagal and Kadu (1990) and Lee and Kim (1994) reported a longer interval between GnRH treatment and onset of oestrus. This difference might be attributed to the ovarian status at the time of treatment since plasma oestradiol concentrations were correlated with the degree of LH release in response to GnRH (Kesler *et al.*, 1977; Fernandes *et al.*, 1978). The results obtained with 'Dinofertin' finds agreement with that of Benmrad Stevenson (1986), Young and Anderson (1986) and Al-Raheem and Al-Ritha (1990). This shorter interval from PGF_2 alpha treatment to onset of first exhibited oestrus could be attributed to the rapid uterine involution and early resumption of ovarian activity. On the contrary, Macmillan *et al.* (1987) and Morton *et al.* (1992) reported no significant effect with respect to onset of oestrus after treatment. This might be attributed to the variation in the day of administration of the drug as suggested by Morton *et al.* (1992).

5.4 Intensity of oestrus

Data presented in Table 7 and Fig.5 revealed that in group I, 20, 50 and 30 per cent of animals exhibited high, medium and low intensity of oestrus respectively which in group II, the corresponding values were 10, 40 and 50 per cent respectively. In the control group, on the otherhand, 33.33 and 66.66 per cent of animals showed medium and low intensity of oestrus respectively while none exhibited high intensity of oestrus. However, statistical analysis revealed no significant difference. But it may be noted that in natural oestrus, none of the animals showed high intensity of oestrus compared to experimental animals indicating that 'Receptal' and 'Dinofertin' treatment during early postpartum period resulted in better expression of oestrus. This agrees with the findings of Pattabiraman *et al.* (1986) who noticed that moderate and mild signs of oestrus were more common in animals treated with Receptal. They further reported that about 65 per cent of the cows subjected to administration of "Receptal" showed only moderate or mild signs of oestrus and opined that intense manifestation of oestrus was not a common feature in those treated with "Receptal".

Comparable data on the intensity of oestrus after prostaglandin F_2 alpha treatment during early postpartum period were not available in the literature. But it may be noted that percentage of mild oestrus was more in natural oestrus than in 'Dinofertin' treated animals indicating beneficial effects of 'Dinofertin' in the manifestation of oestrus signs.

The rate of ovulation in cows that exhibited oestrus within 45 d of calving was 90 per cent in group I, 77.77 per cent in group II and 60 per cent in group III (Table 9). Britt (1974) reported good success in inducing ovulation when treated with GnRH in early postpartum cows. Good ovulation rates in cows treated with GnRH were also reported by Manns and Richardson (1976), Garverick *et al.* (1980), Pattabiraman *et al.* (1986), Roche *et al.* (1992) and Crowe *et al.* (1993). However, Madhavan and Raja (1983) reported a poor ovulation rate.

Benmrad and Stevenson (1986) reported that prostaglandin F_2 alpha induced precocious oestrus or ovulation or both and also had potential to control both timing and frequency of these traits for cows postpartum. Similar observation were reported by Young and Anderson (1986) and Young (1989). This may be attributed to the effect of prostaglandin F_2 alpha on uterine involution and early resumption of ovarian activity as reported by Madej *et al.* (1984).

5.4.1 Serum progesterone concentration 10 d after exhibited oestrus

In cows that ovulated within 45 d of calving serum progesterone concentration 10 d after first exhibited oestrus ranged from 3.2 to 6.3 ng/ml (4.76 ± 0.33 ng/ml) and 4.1 to 6.2 ng/ml (5.12 ± 0.31 ng/ml) in group I and II respectively. However, the control animals had a significantly lower concentration of 2.7 to 3.0 ng/ml (2.86 ± 0.09 ng/ml) than treatment groups. This variation might be on account of the possible beneficial effect of the drugs in the formation of a well developed corpus luteum (Thatcher and Wilcox, 1973).

5.5 Interval from calving to first insemination

Perusal of data in Table 11 and Fig.7 revealed that animals in Group I and II, the interval from calving to first insemination ranged from 48 to 62 d (56 ± 1.99 d) and 47 to 61 d (52 ± 1.24 d) respectively. However, in control animals, the interval from calving to first insemination was 54 to 84 d (65.77 ± 2.90 d). Statistical analysis revealed significant difference between treatment and control groups in the interval from calving to first insemination. But between group I and II, there was no significant difference.

These findings are in agreement with the reports by *Carter et al. (1980), Cavestany and Foote (1985), Aboul-Ela* and El-Keraby (1986), Benmrad and Stevenson (1986) and Pattabiraman *et al.* (1986) for GnRH and Young *et al.* (1984) and Young and Anderson (1986) for PGF_2 alpha. The shorter interval from calving to first insemination could be attributed to the beneficial effects of GnRH and PGF_2 alpha in initiating early ovarian activity. However, a longer interval from calving to insemination was reported by Manns and Richardson (1976), Britt *et al.* (1977), Nash *et al.* (1980), Zaied *et al.* (1980) and Stevenson and Call (1988) for GnRH and Richardson *et al.* (1983), Etherington *et al.* (1984), Etherington *et al.* (1988), Armstrong *et al.* (1989) and Morton *et al.* (1992) for PGF_2 alpha. This variation might be on account of a longer interval from calving to detected oestrum.

5.6 Conception rate

The conception rate in the first insemination and overall conception rate in the experimental and control groups are presented in Table 12 and Fig.8. In group I, the first insemination conception rate and overall conception rate was 30 and 90 per cent respectively as against 20 and 70 per cent in group II. In group III (control), the respective values were 11.1 and 55.55 per cent. It may be noted that the first insemination conception rate and overall conception rate were higher in 'Receptal' and 'Dinofertin' treated cows than that in control group. The number of inseminations per conception was 1.7 in group I and 2.25 in group II as against 2.8 in control animals. The results incurred with 'Receptal' finds agreement with that reported by Pattabiraman *et al.* (1986). Nash *et al.* (1980) also opined that injection of 250 μg of GnRH to lactating dairy cows might be useful in increasing the fertility. Kodagali *et al.* (1981) and Benmrad and Stevenson (1986) also reported similarly. Archbald (1990) also stated that GnRH can be effectively administered 14 days after calving for induction of early ovarian cyclicity with satisfactory fertility. On the contrary, Khurana *et al.* (1982) observed a poor conception rate and attributed this to poor ovarian response of cows in early postpartum period and also due to mild subclinical infection of the uterus. However, Heuwieser *et al.* (1994) stated that efficacy of GnRH with regard to fertility might be consistent in all parities and body condition groups.

The conception rate obtained with 'Dinofertin' in this study agrees with that reported by Benmrad and Stevenson (1986) who indicated improved fertility associated with an increased frequency of oestrus and ovulation during postpartum period. The results are favourably corroborative to the findings of Young *et al.* (1984) who obtained first service conception rate of 68 per cent when single 25 mg Dinoprost was administered during the period from 14 to 28 d after calving to 43 per cent of the untreated controls. Benmrad and

Stevenson (1986) and Young and Anderson (1986) also reported advancement in the resumption of ovarian activity and improved conception for first service by Dinoprost injection soon after calving. In contrast, Richardson *et al.* (1983) found no significant difference in the administration of prostaglandin over controls in the interval from parturition to service and conception rate and concluded that in the absence of abnormalities of the reproductive tract, there was no economical advantage in using GnRH - prostaglandin F_2 alpha regimen postpartum with dairy cattle.

5.7 Interval from calving to conception

Perusal of data in Table 13 and Fig.10 revealed that in group I and II, the interval from calving to conception ranged from 61 to 95 d (69.77 ± 3.70 d) and 61 to 103 d (75.8 ± 5.62 d) respectively. However, in control group, the interval ranged from 66 to 108 d with a mean of 95 ± 6.04 d. Statistical analysis revealed significant difference between treatment and control groups. The results incurred in this aspect is in agreement with Thatcher and Wilcox (1973) who observed that as the number of oestrous periods before 60 days postpartum increased, services per conception and interval to conception decreased. Britt (1974) observed better fertility and reduced interval to conception in animals that began cyclic activity earlier than they normally would. Bosu (1982) reported that fertility of dairy cows during the normal breeding was in direct proportion to the number of oestrous cycles prior to breeding. He opined that fertility increased with each ovulation postpartum. Stevenson and Call (1983) observed improved first service conception rates and decreased interval to conception when cows had one or two heats by day 40 and were first inseminated before day 60 postpartum.

5.8 Early pregnancy diagnosis from serum - progesrerone concentration

Twenty animals were declared pregnant on the basis of higher serum progesterone concentration on day 20 (7.06 ± 0.25 ng/ml) than on day 10 (5.985 ± 0.22 ng/ml) after insemination and seventeen non-pregnant on the basis of low serum progesterone concentration on day 20 (1.576 ± 0.14 ng/ml) than on day 10 (5.806 ± 0.24 ng/ml) after insemination. The accuracy of prediction was 70 per cent for pregnancy and 100 per cent for non-pregnancy. The results obtained are favourably corroborative to the findings of earlier workers like Wishart *et al.* (1975), Pennington *et al.* (1976), Agarwal *et al.* (1977), Roche *et al.* (1985), Robinson *et al.* (1989) in cows and Perera *et al.* (1980), Singh *et al.* (1980), Chauhan and Sharma (1983) in buffaloes. It might be attributed to the fact that serum progesterone concentration showed a similar trend of increase upto day 15 in both pregnant and non-pregnant cows after which it declined in non-pregnant to values as low as at the time of oestrus and increased till day 21 in pregnant cows. However, the over estimation of pregnancy rate prediction by this technique might be due to embryonic death or irregular cycles as reported by Pope *et al.* (1976).

By adopting treatment with GnRH analogue or PGF_2 alpha, it was possible to bring down the interval from calving to first insemination to an average of 56 and 52 days respectively and the calving to conception interval to an average of 69.77 and 75.8 days respectively, making it possible to obtain an annual calf crop of one. Considerable reduction in the dry period coupled with the annual calf crop of one makes these treatments cost effective. It is further concluded that the accuracy of pregnancy diagnosis can be improved by progesterone assay on day 20 and 30, instead of 10 and 20 after insemination, as early embryonic loss can be detected on assay at day 30.

Summary

SUMMARY

The objective of the present investigation was to evaluate the efficacy of administration of gonadotrophin releasing hormone and prostaglandin F_2 alpha for early induction of oestrus and fertility during early postpartum period.

The material for the present study consisted of 30 crossbred cows belonging to Livestock Research Station attached to Kerala Agricultural University. These cows which had normal parturition were allotted into three different treatment groups. Ten animals in group I were administered "Receptal" 5 ml intramuscularly once on 14th day of calving. Ten animals in group II were administered "Dinofertin" 5 ml intramuscularly once on 14th day of calving. Ten animals in group III were administered normal saline, 5 ml intramuscularly once on 14th day of calving and formed the control.

Results obtained and inferences drawn are summarised below. The time taken for regression of pregnancy corpus luteum averaged 14.5 ± 0.37, 14.9 ± 0.45 and 15.3 ± 0.87 days for the three groups respectively. In group I, II and III, uterine involution was complete in 25.3 ± 0.47 d (23 to 27 d), 25 ± 0.77 d (22 to 29 d) and 34.6 ± 1.79 d (28 to 46 d)

respectively. This variation was significant between treatment and control groups. However, the difference was not significant between the two treatment groups. Analysis of data revealed that pluriparous cows took significantly longer period for uterine involution than primiparous cows. Time taken for the first exhibited oestrus after calving ranged from 27 to 44 d (32.1 ± 1.69 d), 26 to 48 d (33.5 ± 1.93 d) and 34 to 64 d (46.1 ± 3.19 d) respectively. Significant difference was observed in the interval from calving to first oestrus between animals in treatment and control group. In group I, all the animals treated with `Receptal' evinced oestrus within 45 d of calving, while in group II, out of ten cows treated with `Dinofertin', nine exhibited oestrus. In group III (control) out of ten cows, only 5 evinced oestrus within 45 d of calving, the difference being significant statistically. Time taken for onset of oestrus after treatment ranged from 13 to 24 d (18.1 ± 1.69 d), 12 to 3 4 d (19.5 ± 1.93 d) and 20 to 50 d (30.7 ± 3.37 d) respectively. Analysis of data revealed that the time taken for onset of oestrus after treatment, was prolonged in animals of control than treatment groups. In group I, 20 and 50 per cent of cows exhibited high and medium intensity of oestrus while in group II, the corresponding figures were 10 and 40 percent. In the control group, none of the cows exhibited high intensity of oestrus while 33.33 per cent exhibited medium intensity of oestrus. The ovulation rate in cows that exhibited oestrus upto 45 d of calving was 90, 77.77 and 60 per cent respectively for the groups I, II and III respectively. In the ovulated cows, serum progesterone concentration 10 d after first exhibited oestrus, within 45 d of calving, ranged from 3.2 to 6.3 ng/ml (4.76 ± 0.33 ng/ml), 4.1 to 6.2 ng/ml (5.12 ± 0.31 ng/ml) and 2.7 to 3.0 ng/ml (2.86 ± 0.09 ng/ml) respectively for the three groups. Analysis of data revealed that serum progesterone concentration in cows ovulated among the treatment groups was higher than in control group. The interval from calving to first insemination in group I, II and III ranged from 48 to 62 d (56 ± 1.99 d), 47 to 61 d (52 ± 1.24 d) and 54 to 84 d (65.77 ± 2.90 d) respectively. Analysis of data revealed that the interval to first insemination after calving was significantly longer in control than treatment groups. In group I, the first insemination conception and overall conception rates were 30 and 90 per cent, as against 20 and 70 per cent in group II. In the control group, the respective figures were 11.1 and 55.55 per cent. The percentage of conception, however, in the experimental group was higher than the control group. The number of inseminations per conception was 1.7 in group I, 2.25 in group II and 2.8 in group III. The animals in group I and II required comparatively fewer number of inseminations per conception than control. In group I, the interval from calving to conception averaged 69.77 ± 3.70 d (61 to 95 d) as against 75.8 ± 5.62 d (61 to 103 d) in group II. However, the control animals took significantly longer interval of 95 ± 6.04 d (66 to 108 d) for conception after calving. Early postpartum treatment with GnRH and prostaglandin F_2 alpha not only helped in reducing the service period, but also was cost-effective taking into consideration the cost for maintenance of the animal with each day prolongation of dry period than normal. Out of twenty cows predicted pregnant, on the basis of higher serum progesterone on day 20 after insemination (mean 7.06 ng/ml), fourteen were confirmed pregnant (70%) by rectal palpation 45 d after insemination. All the seventeen cows predicted non-pregnant on the basis of lower serum progesterone concentration on day 20 (mean 1.576 ng/ml) were confirmed non-pregnant (100%) by rectal palpation on day 45 after insemination.

To sum up it may be stated that gonadotrophin releasing hormone or prostaglandin F_2 alpha could be successfully used for induction of early postpartum oestrus, thereby reducing the calving to conception interval and AI index and therefore is cost-effective. Further, it may be concluded that the accuracy of pregnancy diagnosis can be improved if serum progesterone assay is carried out on days 20 and 30 instead of day 10 and 20 after insemination.

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FERTILITY MANAGEMENT OF EARLY POST - PARTUM COWS WITH GONADOTROPHIN RELEASING HORMONE AND PROSTAGLANDIN F_2 ALPHA

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ABSTRACT OF A THESIS

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ABSTRACT

With the object of evaluating the efficacy of gonadotrophin releasing hormone (GnRH) and prostaglandin F_2 alpha (PGF_2 alpha) for induction of early postpartum oestrus and reduction of calving to conception interval, 30 crossbreds cows which had normal parturition, selected from Livestock Research Station, Thiruvazhamkunnu were allotted to three different treatment groups. Ten cows each in group I, II and III were administered intramuscularly 5 ml Receptal, 5 ml Dinofertin and 5 ml Saline respectively on 14th day of calving. The time taken for regression of pregnancy corpus luteum averaged 14.5 ± 0.37, 14.9 ± 0.45 and 15.3 ± 0.87 days respectively in the three groups. Uterine involution was complete in 25.3 ± 0.47, 25.0 ± 0.77 and 34.6 ± 1.79 days respectively. Analysis of data revealed significant variation in the uterine involution between experimental and control groups. The interval from treatment to onset of oestrus was 18.1 ± 1.69, 19.5 ± 1.93 and 30.7 ± 3.37 days respectively for the three groups and the interval from calving to first exhibited oestrus was 32.1 ± 1.69, 33.5 ± 1.93 and 46.11 ± 3.19 days respectively. Statistical analysis revealed significant variation in the interval from calving to first oestrus and treatment to onset of oestrus between treatment and control groups. Percentage of cows that evinced oestrus

within 45 d of calving were 100, 90 and 50 respectively in the three groups. This variation between treatment and control groups was statistically significant. A higher proportion of cows from group I and II showed medium to high intensity of oestrum when compared to control. The ovulation rate in cows that exhibited oestrus upto 45 d of calving was 90, 77.77 and 60 per cent respectively in group I, II and III. There was significantly higher progesterone level in the ovulated cows of the treatment groups than that of control. The interval from calving to first insemination in group I, II and III were 56 ± 1.99, 52 ± 1.24 and 65.77 ± 2.90 days respectively and the interval from calving to coneption were 69.77 ± 3.70, 75.87 ± 5.62 and 95.0 ± 6.04 days respectively. The variations in service period and calving to conception interval between treatment and control groups was statistically significant. The first insemination conception and overall conception rate with three or more A.I. were 30 and 90 per cent for group I, 20 and 70 per cent for group II and 11.1 and 55.5 per cent for group III. The A.I. index was 1.7, 2.25 and 2.8 for the three groups respectively. Eventhough, there was no significant difference in the first insemination conception and A.I. index between the three groups, there was apparently better conception rate in the treatment groups with reduction in A.I. index. However, no significant variation in any of the reproductive parameters between the two treatment groups was noticed. The accuracy of prediction of pregnancy by progesterone assay on day 20 was only 70 per cent as against 100 per cent for non-pregnant animals. The accuracy of pregnancy diagnosis by this method can be improved by a second assay on day 30, which will cover loss of pregnancy due to early embryonic death.

It is concluded that GnRH or PGF_2 alpha administered on the fourteenth day of calving will help early induction of oestrum and conception and is therefore cost-effective.