SYNCHRONIZATION OF OVULATION AND TIMED ARTIFICIAL INSEMINATION TO IMPROVE FERTILITY IN POSTPARTUM DAIRY COWS

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DECLARATION

I hereby declare that the thesis entitled "**SYNCHRONIZATION OF OVULATION AND TIMED ARTIFICIAL INSEMINATION TO IMPROVE FERTILITY IN POSTPARTUM DAIRY COWS"** is a bonafide record of research work done by me during the course of research and that this 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 the thesis, entitled **"SYNCHRONIZATION OF OVULATION AND TIMED ARTIFICIAL INSEMINATION TO IMPROVE FERTILITY IN POSTPARTUM DAIRY COWS"** is a record of research work done independently by **Dr. Rajeswari. T,** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, associateship or fellowship to her.

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Introduction

1. INTRODUCTION

India has vast resource of livestock and poultry, which play a vital role in improving the socioeconomic conditions of rural masses. About 22.45 million people work in livestock sector which is 5.5 per cent of the total working population of the country. The contribution of livestock and fisheries sector to the total GDP during 2006-07 was 5.26 per cent. Though India ranks first in respect of cattle and buffaloes, yet in terms of productivity, India's huge livestock resources have to improve much to achieve the standards envisaged. The poor productivity as well as the quality of production, therefore, remains a cause of concern in livestock sector.

Kerala is an agricultural state and a majority of population is engaged in agriculture, animal husbandry and allied occupations. Livestock and poultry rearing contribute a major share of subsidiary income. The total cattle population of Kerala as per 2003 livestock census was 21 lakhs which showed a drastic decline of 14.79 per cent from last census.

Reproductive efficiency is often a limiting factor in dairy herd productivity and profitability. In this context reproductive biotechnology as a tool for improving the productive and reproductive efficiency of livestock is gaining momentum during the last few years. Research in this field has led to the development of assisted reproductive technologies such as artificial insemination, oestrus synchronization, embryo transfer, in vitro fertilization, embryo culture, oocyte, embryo and sperm cryopreservation, which have been used successfully to make genetic improvement of livestock resulting in increased milk, meat and fiber production. During the past, intense genetic selection in cattle has been made for increased milk yield in most of the developing countries. While milk yield has almost doubled during this period, the fertility in dairy cows is reported to have decreased with services per conception and days open being increased. Physical

changes resultant to the genetic selection for high milk production has made cows more susceptible to factors for reduction in fertility.

The first assisted reproductive technology (ART) developed was artificial insemination (AI), which is an important technique for the genetic improvement of animals, as a few select males can produce sufficient sperm to inseminate thousands of females. The benefits of using a breeding system that incorporates oestrus synchronization and AI will allow producers to reach certain production or economic goals quicker than natural service.

Increased milk production has led to a decline in the reproductive performance of dairy cows due to a prolonged inter calving period. A 12 to 13 month calving interval is recommended to be optimal for high annual milk yield and economic worth to dairy producers. Several factors such as delayed resumption of normal ovarian cyclicity following parturition, poor oestrus detection, silent oestrus, improper timing of insemination, reduced conception rate and ovarian disorders during early postpartum period are the major problems, which decrease the reproductive efficiency in a dairy farm and have contributed to a prolonged intercalving interval and reduced profitability in dairy farming. Majority of high yielding early post partum cows suffer from one or another ovarian disorder, regular cyclicity before 50 days post partum is observed only in 51 per cent of animals (Lopez-Gatius, 2002).

Oestrus detection is definitely a time and labour consuming process, which makes artificial insemination programmes difficult for large herds. Oestrus detection can be eliminated by breeding animals at a designated or pre-determined time, also known as timed artificial insemination (TAI). Similarly oestrus synchronization systems that incorporate TAI have advanced in their ability to control the oestrous cycle and induce ovulation at a pre-determined time. Pregnancy rates to TAI have matched or exceeded pregnancy rates to AI and twice daily heat detection, allowing producers the opportunity to incorporate these

technologies without too much time and labour involved. Oestrus synchronization along with AI can be a valuable tool for smaller operations where the number of cattle may not justify the cost for purchasing a bull of superior quality and genetic make up. Treatments aimed at synchronizing oestrus or inducing ovulation allow for the effective management of TAI in lactating dairy cows without the need for detecting oestrus. Today, oestrus synchronization and AI remain to be the most popular and widely applicable reproductive biotechnology available in veterinary practice.

In order to improve the oestrus detection rate, the oestrus synchronization programmes using Prostaglandin $F_2\alpha$ ($PGF_2\alpha$) that focus on controlling the life span of the corpus luteum (CL) have been implemented (Lucy *et al.,* 1986). However oestrus was not synchronized precisely with $PGF_2\alpha$ as this treatment does not synchronize growth of follicles but only regulates the life span of CL. A timed artificial insemination protocol (Ovsynch) based on the use of gonadotrophin releasing hormone (GnRH) and $PGF_2\alpha$ to synchronize oestrus and ovulation was developed for use in dairy cows (Pursley *et al.,* 1995). This protocol synchronizes both follicular wave development and regression of CL.

The scenario clearly shows the need for an in-depth study of oestrus synchronization programmes and fixed timed artificial insemination (FTAI) in early post partum dairy cows, so as to evolve and recommend a simple, flexible, better and more consistent oestrus synchronization programme without compromising on pregnancy rates. Hence the present study was undertaken with the objective of comparing the efficacy of different regimes for oestrus and ovulation control using $PGF_2\alpha$ and GnRH to improve fertility in postpartum dairy cows.

Review of literature

2. REVIEW OF LITERATURE

2.1 FOLLICULAR DYNAMICS IN DAIRY CATTLE

Savio *et al.* (1990) observed that after parturition first dominant follicle in many cows ovulated during 3 to 5 weeks postpartum. Roche *et al.* (2000) noticed antral follicular development between day 15 and 35 postpartum but in majority of normal cows first ovulation occurred without behavioural oestrus due to the refractoriness of the brain induced by high levels of oestradiol during late gestation. Resumption of normal cyclicity is preceded by the development of a short-lived CL. The major limiting factor to ovulation was the reinitiation of adequate LH secretion in the form of circhoral LH pulses to support final follicular maturation and subsequent ovulation of a dominant follicle (Stevenson, 2001).

Diskin *et al.* (2002) established that a wave of follicular development in cattle was characterized by the synchronous growth of a number of small follicles followed by selection of a dominant follicle and subsequent regression of subordinate follicles. Each wave had an inherent life span of 7 to 10 days as it progressed through recruitment, selection, dominance, atresia or ovulation. It was well established that two or three follicular waves occurred in majority of bovine oestrous cycles (Driancourt, 2000; Day and Geary, 2005).

The period of time from parturition to the first postpartum oestrus accompanied by ovulation in the cow was found to be as short as 15 days or longer than 100 days and the usual postpartum interval being 60 to 90 days (Shrestha *et al.*, 2004). They further observed that delay in resumption of postpartum ovarian cyclicity was more in high producing dairy cows. The reasons attributed were prolonged luteal phase and delayed first ovulation.

2.2 OESTRUS SYNCHRONIZATION IN DAIRY CATTLE

Oestrus control strategies were mainly based on induction of ovulation with GnRH or its agonists, controlling the life span of CL with prostaglandins or prevention of oestrus using progestagens. The target for a successful oestrus synchronization treatment was precise control of onset of oestrus together with high fertility at synchronized oestrus (Driancourt, 2000). Pharmacological control of the oestrus cycle involved synchronization of follicular development, control of CL regression and synchronization of ovulation (Thatcher *et al.,* 2001).

Stevenson (2001) opined that with an expected waiting period of 50 days, oestrus and conception rate of 65 per cent, an average calving interval of 365 days was attainable in dairy herds.

Diskin *et al.* (2002) followed three approaches for controlling the ovarian activity and to regulate the oestrus cycle in dairy herds. (1) Use of luteolytic agent $PGF_2\alpha$ alone or any of its analogues (2) Administration of exogenous progesterone, progestagen treatment combined with the use of exogenous oestradiol or GnRH to control new follicular wave emergence and to shorten the life span of CL (3) Prior follicular wave synchrony followed by induced luteolysis.

Optimizing the first postpartum pregnancy rate was important to improve reproductive efficiency in dairy herds for maximum productivity (Santos *et al.*, 2003).

The fundamental component of all timed insemination protocols was to synchronize the waves of follicular development so that all females had a dominant follicle of same age and size (Day and Geary, 2005).

2.2.1 Hormones Used in Oestrus Synchronization

An ovulation synchronization protocol involving GnRH and $PGF_2\alpha$ (Ovsynch) was developed for use in dairy cows (Pursley *et al.,* 1995). Alternatively few other researchers used various other estrous synchronization protocols with preparations of gonadotropins especially equine chorionic gonadotropin (eCG) and human chorionic gonadotropin (hCG).

In a review on this chapter, Xu and Burton (1999) confirmed that progesterone, GnRH, oestrogen, $PGF_2\alpha$ and a combination of oestrogen and progesterone were needed to synchronize the onset of follicular waves in cattle and further opined that successful estrous synchronization programmes should aim to synchronize follicular wave development as well as onset of oestrus and ovulation.

2.2.1.1 Gonadotrophin Releasing Hormone

Administration of GnRH at any stage of oestrus cycle increased luteinising hormone (LH) and follicle stimulating hormone (FSH) in the peripheral circulation with in 2 to 4 hours (Chenault *et al.,* 1990; Stevenson *et al*., 1993), which altered the pattern of growth of the existing follicle with the follicle response being dependent on the stage of the follicular wave at the time of treatment. GnRH administered after dominant follicle selection caused ovulation and induced a new wave emergence 1 to 2 days later while GnRH administered before selection had no effect on the progression of the existing follicular wave (Diskin *et al.,* 2002).

Treatment with buserelin induced the resumption of cyclic ovarian activity in postpartum anoestrus cows as determined by increased serum progesterone concentration and fertility rate during induced oestrus which was comparable to that of cyclic cows (Twagiramungu *et al.,* 1992a, b).

According to Twagiramungu *et al.* (1995a) and Ryan *et al.* (1998) GnRH treatment resulted in ovulation of largest follicle with subsequent formation of a new CL or its regression by atresia depending on the stage of development which resulted in decreased concentration of oestradiol and recurring oestrus was prevented between day 0 and day 6. Further they observed that if CL was present at the time of GnRH treatment, LH increased the number of large luteal cells. The FSH increased the turn over of follicles from class I to class II, but increased atresia in class II limiting further growth to large sized follicles. Within 3 to 4 days after GnRH treatment a new dominant follicle was selected from the newly synchronized follicular wave.

2.2.1.2 Oestrogen, Progestagens and Their Combinations

The insertion of progesterone releasing intravaginal device (PRID) without oestradiol capsule, during the luteal phase, increased conception in cows with low plasma progesterone, and decreased conception in cows with high plasma progesterone. The highest conception rate was obtained when endogenous progesterone was measured and appropriate exogenous agent was applied (Folman *et al.,* 1990).

According to Nasalaji *et al.* (1996) most oestrus synchronization programs in cattle aimed the use of progestagens and oestrogens to synchronize follicular wave emergence in order to enhance the tightness of oestrus synchrony. Oestrogens played an important role in several reproductive events that occur around the time of oestrus such as triggering LH surge and ovulation, luteolysis and oestrus behaviour. However, oestrogens could produce adverse effects such as ovarian cyst and false oestrus behaviour if it was administered in the absence of progesterone. The atretogenic effect of oestrogen and progesterone on ovarian follicles was used to synchronize follicular wave emergence. Exogenous progestagens control expression of oestrus, occurrence of preovulatory LH surge and ovulation (Nasalaji *et al.,* 2001).

The discovery that oestrogens were luteolytic, when administered during the early part of the oestrus cycle led to the incorporation of oestrogens as part of the synchronization treatment (Diskin *et al.,* 2002). This in turn facilitated a reduction in the duration of progestagen based synchronization treatments from 18 to 20 days to 9 to 12 day duration. This resulted in increased precision of oestrus onset and significant improvement in pregnancy rate compared with progestagen treatment for more than 14 days and most protocol required $PGF_2\alpha$ as luteolytic agent.

Increased exposure of cows to endogenous oestrogens or administration of oestrogens, increased the incidence of oestrus which enhanced fertility (Pancarci *et al.,* 2002; Stevenson *et al.,* 2004).

Bo and Mapletoft (2004) reported that oestrogens often required three to five days, progesterone two to five days and GnRH two days for initiation of a new follicular wave.

Combining presynchronization with progesterone to the Ovsynch protocol increased the percentage of cows with elevated progesterone concentrations at the time of $PGF_2\alpha$ just before TAI, increased the proportion of cows in early dioestrus at the start of the Ovsynch protocol and consequently increased pregnancy rate after TAI (El-Zarkouny *et al.,* 2004).

Oestrogen was used to increase the synchrony of oestrus in $PGF_2\alpha$ based oestrus synchronization programs (Martinez *et al.,* 2004). Treatment with 0.4 mg oestradiol benzoate 40 to 48 h after $PGF_2\alpha$ administration in cyclic beef cows reduced the variation in the intervals to LH release and onset of oestrus.

According to Saldarriga *et al.* (2007) the use of a controlled internal drug release (CIDR) device containing progesterone in combination with GnRH,

$PGF_2\alpha$ and TAI was a relatively convenient and systematically functional synchronization protocol.

The combination of oestradiol cypionate (ECP) and prostaglandin - GnRH - prostaglandin enhanced oestrus and induced ovulation, and it was potentially developed as a new method to routinely synchronize oestrus and ovulation in dairy cows (Amer, 2008).

2.2.1.3 Equine Chorionic Gonadotrophin (eCG), Human Chorionic Gonadotrophin (hCG) and Their combinations

The simultaneous administration of hCG and oestradiol benzoate 12 hours after treatment with prostaglandins in dairy cows and heifers with a mature CL was able to shorten the mean time to onset of oestrus and increased the precision in ovulation (Lopez-Gatius and Vega-Prieto, 1990).

The norgestamet/hCG regimen altered the development and regression of CL together with ovulation of a newly recruited dominant follicle. This protocol could further be developed for oestrus synchronization programs to consistently achieve close synchrony of oestrus and high fertility in cattle (Nasalaji *et al.,* 1996).

Both GnRH and hCG were effective in inducing accessory CL but the subsequent increase in progesterone concentrations was greater in hCG treated heifers and dairy cows (Diaz *et al.,* 1998). According to them the longer half life of hCG probably provided a longer period of LH like stimulation of the follicle and luteotropic action on the developing accessory and natural CL.

In cattle, treatment with hCG on day 5 and/or day 7 could increase progesterone concentrations by enhancing secretion from the existing CL and also by inducing ovulation and formation of an accessory CL (Santos *et al.,* 2001).

2.2.1.4 Prostaglandin $F_2\alpha$

The mechanism of luteolysis after administration of $PGF_2\alpha$ was described by various researchers (Lauderdale *et al*., 1974; Odde, 1990). Administration of $PGF_2\alpha$ resulted in immediate regression of CL and rapid decline of blood progesterone levels to basal concentrations within 24 h (Harrison *et al.,* 1985). This in turn led to increased LH pulse frequency, causing a significant increase in the oestradiol from the dominant follicle. The oestradiol progesterone ratio changes modified secretory pattern of gonadotrophins leading to the development of an ovulatory follicle (Diskin *et al.,* 2002; Day and Geary, 2005).

In response to $PGF_2\alpha$ induced luteolysis, synchrony of LH surge depended on the population of large follicles at the time of treatment (Sirois and Fortune, 1990). $PGF_2\alpha$ administration was effective if cows and or heifers had a functional CL and were in 5 to 18 days of oestrus cycle. Further it was observed that $PGF_2\alpha$ was ineffective on noncycling cows and immature heifers (Sirois and Fortune, 1988).

2.3 PROSTAGLANDIN AND ITS ANALOGUES TO IMPROVE FERTILITY IN DAIRY CATTLE

2.3.1 Prostaglandins for Oestrus Synchronization

$PGF_2\alpha$ was extensively used as a therapeutic agent for tackling postpartum ovarian disorders in cattle (Seguin, 1980; Pankowski *et al.,* 1995) and as a drug for oestrus synchronization (Odd, 1990; Nebel and Jobst 1998; Thatcher *et al.,* 2001).

In single $PGF_2\alpha$ protocol after the administration of the drug, breeding those at detected oestrus for 5 days was the usual practice followed (Lauderdale, 1980) or detection of oestrus and AI for 4 days and administering those that were

not detected in oestrus on day 5 and breeding from day 5 to through 9 by detection of oestrus (Odde, 1990).

A number of different protocols were developed to use $PGF_2\alpha$ in breeding program schedules. A double $PGF_2\alpha$ protocol involving administration of the drug 11 to 14 days apart seemed to be capable of bringing most cows to oestrus (Odde, 1990). The 14 day rather than 11 day was found to be giving improved conception rate because most cows will be in the late luteal phase of the cycle when they received second $PGF_2\alpha$ dose (Murugavel *et al.,* 2003).

The prostaglandins along with other hormones were tried for therapeutic oestrus synchronization in postpartum dairy cows with various ovarian disorders (Bartolome *et al.,* 2000; Lopez-Gatius *et al.,* 2001; Pursley *et al.,* 2001; Lopez-Gatius and Lopez-Bejar, 2002).

2.3.2 Fertility Following $PGF_2\alpha$ Induced Oestrus in Dairy Cattle

Various researchers reported synchronization and conception rates of 80 to 85 and 50 to 80 per cent respectively in response to oestrus synchronization using single $PGF_2\alpha$ protocol (Oxender *et al*., 1974; Duetscher *et al*., 1982; Jacob *et al*., 1995; Kharche and Srivastava 2005).

Seguin (1980) conducted a trial for oestrus synchronization in cows using double PGF2α protocol and obtained a conception rate of 22 per cent after TAI, whereas Young and Henderson (1981) found no significant difference in conception rate compared to control.

McIntosh *et al*. (1984) reported improved conception rate when animals were treated with cloprostenol and inseminated on observed oestrus. The time of onset of oestrus was influenced by the stage of oestrus cycle at the time of $PGF_2\alpha$ treatment. Due to varying oestrus onset times improved conception rates were

obtained after AI at detected oestrus rather than FTAI (Lucy *et al.*, 1986; Stevenson *et al.,* 1987; Archbald *et al.,* 1992; Xu *et al.,* 1996; Mialot *et al.*, 1999; Murugavel *et al.,* 2003). Several researchers reported normal or above normal fertility following oestrus synchronization with $PGF_2\alpha$ compared to control (Plunkett *et al*., 1984; Lucy *et al.,* 1986).

Hansen *et al*. (1987) reported that incomplete luteal regression following $PGF_2\alpha$ resulted in elevated serum progesterone during oestrus, which altered oestrus and ovulation. They opined that impaired corpus luteum function was one of the explanations for the low fertility following $PGF_2\alpha$ induced oestrus in cattle.

Gay and Upham (1994) administered $PGF_2\alpha$ to clinically normal cows with a palpable corpus luteum 20 to 40 days postpartum and reported lowered conception rate at first breeding where as Heuweiser *et al.* (1997) and Tenhagen *et al.* (2000) reported that reproductive performance in dairy cattle was improved following double $PGF_2\alpha$ protocol with out assessing ovarian status when compared with a single dose based on detecting a CL by rectal palpation or milk progesterone by radio immuno assay (RIA.)

Ajitkumar *et al*. (1996) subjected cross bred heifers of breedable age to prostaglandin therapy at the luteal phase of oestrous cycle and reported first service conception rate and overall conception rate were 33.33 per cent and 66.67 per cent respectively where as Leeba (2003) obtained first service conception rate of 42.86 and 50 per cent in cows and heifers respectively.

2.3.3 Prostaglandin Treatment During Early Post Partum Period

Administering $PGF_2\alpha$ during the early postpartum period resulted in increased first service conception rate and reduced inter calving interval in dairy cows (Thatcher and Wilcox, 1973; Young *et al.,* 1984; Benmrad *et al.,* 1986; Wensel *et al.,* 1995). Similarly Burton and Lean (1995) noticed significant

reduction in the number of days open but could not obtain increased first service conception rate.

According to Young *et al*. (1984), the resumption of ovarian cyclicity after calving could be advanced by the administration of $PGF_2\alpha$ or its synthetic analogues.

White and Dobson (1990) reported that $PGF_2\alpha$ was capable of improving the reproductive performance of dairy cows when given before the end of voluntary waiting period (VWP).

2.3.4 Factors Influencing the Effect of Prostaglandin Treatment

2.3.4.1 Stage of Oestrus Cycle at the Time of Prostaglandin Treatment

An enhanced oestrus response and normal fertility were reported when $PGF_2\alpha$ was given at the late, rather than early to middle stage of luteal phase in heifers (Tanabe and Hann, 1984) and in dairy cows (Rosenberg *et al.,* 1990; Kristula *et al.,* 1992; Lucy *et al*., 1992; Ferguson and Galligan, 1993; Xu *et al.,* 1996).

The mean interval to oestrus was 48 to 72 hours when $PGF_2\alpha$ was administered on oestrus cycle day 5 to 8 (Tanabe and Hann, 1984; Dailey *et al*., 1986; Ferguson and Galligan, 1993). According to Watts and Fuquay (1985) $PGF_2\alpha$ administration during midcycle or later in the luteal phase resulted in a mean time to oestrus of 70 and 62 hours respectively (King *et al.,* 1982; Stevenson *et al.,* 1984).

Double $PGF_2\alpha$ protocol at 14 day interval seemed to show an improved response over the 11 day protocol because two injections given 14 days apart ensured that most animals were in the late luteal stage when they received the

second $PGF_2\alpha$ injection (Young, 1989; Folman *et al.,* 1990; Rosenberg *et al.,* 1990).

2.3.4.2 Stage of Follicle at the Time of Prostaglandin Administration and Variation in the Duration of Onset of Oestrum

There was considerable variation in the interval from $PGF_2\alpha$ treatment to oestrus and ovulation. This variability could be attributed to the status of the follicular wave at the time of treatment (Kastelic *et al.,* 1990; Twagiramungu *et al.,* 1992b, 1995a; Ferguson and Galligan, 1993; Adams, 1994).

Kastelic and Ginther (1991) reported that the time from $PGF_2\alpha$ administration to ovulation was dependent on the maturity and size of the most emergent dominant follicle, because a small dominant follicle takes longer to grow into an ovulatory follicle.

2.3.4.3 Effect of Progesterone Level on Synchronized Oestrus

The conception rate in cows following $PGF_2\alpha$ induced oestrus was positively correlated with plasma concentration of progesterone that reached during the days preceding the luteolysis (Chenault *et al.,* 1976; Jaster *et al.,* 1982; Dailey *et al.* 1986; Lucy *et al.*, 1986; Folman *et al.,* 1990; Stevens *et al.,* 1993; Birnie *et al.,* 1997).

Folman *et al.* (1990) and Rosenberg *et al.* (1990) reported that the number of primiparous cows conceived following administration of $PGF_2\alpha$ at 14 day interval was significantly more than cows administered $PGF_2\alpha$ at 11 day interval due to increased level of progesterone prior to ovulation. However Gyawu *et al.* (1991) showed that excessively prolonged period of high progesterone level prior to insemination could suppress fertility.

It was observed that if plasma progesterone levels were higher than 1 ng/ml at the time of first injection of $PGF_2\alpha$, the cycle subsequent to the second injection was longer (Howard *et al*., 1990; Howard and Britt, 1990; Larson and Ball, 1992).

Reports were available which revealed that high progesterone level at the time of administration of $PGF_2\alpha$ was associated with delayed onset of oestrus (Larson and Ball, 1992). Further they observed that oestrus was manifested in cows that had high progesterone concentrations of ≥3.1 ng/ml.

2.3.4.4 Effect of Natural $PGF_2\alpha$ and Synthetic Analogues on Oestrus Response and Fertility

Schams and Karg (1982) found significant difference in the luteolytic action of alfaprostol, cloprostenol, luprostiol and tiaprost in heifers. According to Pursley and Bellow (2007) dinoprost, a natural tromethamine salt of $PGF_2\alpha$ and cloprostenol, a synthetic prostaglandin analogue did not differ in their luteolytic ability but differed in their pharmacokinetic properties when administered to cows. Dinoprost showed a short half life of 7 to 18 minutes whereas cloprostenol was more resistant to endogenous metabolism, maintaining higher circulating concentrations for a longer period of 3 h. Martinez and Thiber (1984) and Seguin *et al.* (1985) opined that there was no difference in fertility in oestrus induced with $PGF_2\alpha$ or its synthetic analogues.

Elmenoufy and Abdou (1989) reported that the oestrus synchronization rate was higher in cows treated with cloprostenol when compared to cows treated with natural $PGF_2\alpha$. On the contrary, Wenzel (1991) reported that a greater proportion of cows with unobserved oestrus showed luteolysis and behavioural oestrus when treated with natural $PGF_2\alpha$ and fenprostalene than cows treated with cloprostenol.

2.3.4.5 Route and Dose of $PGF_2\alpha$ Administration

Many works were conducted to determine the minimum effective dose and the most appropriate route of administration of the drug and administration of reduced dose of $PGF_2\alpha$ at different sites of the reproductive tract namely, intrauterine infusion (Louis *et al*., 1974; Chatterjee *et al.*, 1989; Galina and Arthur, 1990), intravenous (Maurer *et al.*, 1989; Stevens *et al*., 1995), intramuscular (Maurer *et al.*, 1989; Stevens *et al*., 1995), deposition into cervix or vulval lips (Galina and Arthur, 1990), intravulvosubmucosal (Dhande and Cadu, 1994; Colazo *et al.,* 2002a), intraovarian (Berbumez *et al.*, 1999) and subcutaneous (Brogliatti *et al*., 2000; Colazo *et al*., 2002a, b) and through ischiorectal fossa (Colazo *et al.,* 2002b), but the most common route of administration was intramuscular. Moreno *et al.* (1986) reported that injection of 25 mg of dinoprost was sufficient to induce luteolysis in zebu cattle.

Plasma concentration of $PGF_2\alpha$ was raised to maximum level within 10 minutes of exogenous administration and it declined to preinjection level by 90 minutes (Stellflug *et al.,* 1975). Kindahl (1980) opined that prostaglandins having very short half life, when administered and once absorbed into the blood stream would be quickly inactivated by oxidation after one passage through the lungs. Maurer *et al.* (1989) found that $PGF_2\alpha$ administered intravenously would metabolise faster resulting in less peripheral exposure time when compared to other parentral routes. On the contrary, Stevens *et al.* (1995) reported that cloprostenol administered intravenously to non lactating dioestrus dairy cows did not alter the rate of luteolysis, compared to cows given cloprostenol intramuscularly.

2.3.4.6 Effect of Accuracy in Rectal Palpation of CL

Seguin *et al.* (1978) and Dailey *et al.* (1986) reported an error up to 6 per cent during identification of CL by rectal palpation. However Kelton *et al.* (1991) reported that success of oestrus synchronization depended on the accurate identification of a mature CL by rectal palpation. Even though $PGF_2\alpha$ protocols could be employed without screening the ovarian status, gynaecological examination by way of rectal palpation of ovary was often done to detect a mature CL before a single dose of $PGF_2\alpha$ (Wenzel, 1991).

The concentration of progesterone in plasma (Boyd and Munro, 1979), milk (Watson and Munro, 1980) or serum (Mortimer *et al.*, 1983) was used to compare with the accuracy of rectal palpation of CL. Ott *et al.* (1986) reported that identification of CL by rectal palpation was 85 per cent accurate and the major reason for decrease in the success of oestrus synchronization following administration of $PGF_2\alpha$ was due to unreliability of CL palpation by rectal examination.

2.3.5 Fertility in Natural Oestrus Following Synchronized Oestrus

Various researchers reported a significant reduction in fertility in spontaneous natural oestrum following synchronized oestrum (Graves *et al.*, 1974; Plunkett *et al.*, 1984; Stevenson *et al.*, 1984; Tanabe and Hann, 1984; Morrell *et al.,* 1991). Eventhough the physiological events following the administration of $PGF_2\alpha$ were reported to be similar with that of naturally occurring luteolysis (Schultz, 1980; Seguin, 1980), a few authors reported longer time for the occurrence of spontaneous natural oestrus following synchronization of oestrus in cows (Howard *et al.,* 1990; Cardenas *et al.*, 1991; Morbeck *et al.*, 1991).

2.3.6 Oestrus Response, Duration and Intensity of Oestrum After $PGF_2\alpha$ Treatment

Cooper and Furr (1974) administered synthetic analogue of prostaglandin structurally related to "F" series prostaglandins twice at an interval of 12 days and observed that majority of treated Friesian heifers exhibited oestrus 48 to 72 hours after the second treatment. Reports by various researchers revealed that majority of cows treated with $PGF_2\alpha$ exhibited oestrus, two to six days after induction (Dailey *et al*., 1983; Macmillan and Handerson 1984; Seguin *et al*., 1985; Landivar *et al*., 1985).

Johnson (1978) observed that the time of onset of oestrus after first and second injection of a double prostaglandin regime using cloprostenol was 68.6 ± 20.8 and 59.9 ± 15.8 h respectively. The corresponding time interval reported by Reddy *et al*. (2001) was 64.00 ± 10.22 and 52.8 ± 4.07 h respectively.

Nair and Madhavan (1984) reported that the duration of prostaglandin induced oestrus did not show marked variation from the natural oestrus, but observed a high incidence of weak oestrus signs. Similarly Jeba (2005) reported that oestrus response was more pronounced during natural than prostaglandin induced oestrum. On the contrary various researchers observed a marginal increase in oestrus response following administration of $PGF_2\alpha$ when compared to natural oestrum (Ajitkumar, 1994; Jacob *et al*., 1995; Leeba 2003).

Ajitkumar *et al*. (1996) observed that the time taken for induction of oestrus was 63.38 and 67.50 h in single and double prostaglandin regime respectively and the duration of oestrus ranged from 18 to 48 and 24 to 48 h respectively.

Senthilkumar and Rajasekhar (1998) recorded 100 per cent oestrus response to synchronization with 25 mg $PGF_2\alpha$ analogue while only 59 per cent

response to 15 mg of the drug. The time taken for onset of oestrus was 64.92 ± 2.17 and 79.43 ± 3.54 h respectively and the respective mean duration of oestrus was 19.27 ± 1.57 and 22.29 ± 1.48 h.

2.4 EFFECT OF GnRH ON FOLLICULAR DYNAMICS

In cycling cows administration of GnRH and its analogues induced gonadotrophic surge with peak LH within 2 to 3 hours (Foster *et al.*, 1980; William *et al*., 1982; Chenault *et al.* 1990; Evans and Rowlings, 1994) and altered the pattern of follicle growth (Kesler *et al.,* 1980; Thatcher *et al.,* 1989; Wolfenson *et al.,* 1994).

Administration of GnRH during luteal phase caused an alteration of follicular distribution in the ovary by increasing the number of medium sized follicles and decreasing the number of large follicles by inducing luteinisation or formation of a secondary CL following ovulation. (McNatty *et al*., 1981; Skaggs *et al*., 1986; Thatcher *et al.,* 1989; Guibault *et al*., 1990; Stevenson *et al*., 1993). A single injection of GnRH or an agonist was sufficient to induce ovulation or atresia of a dominant follicle (Gaverick *et al.,* 1980; Crowe *et al.*, 1993; Twagiramungu *et al*., 1995b) in cattle.

Several reports demonstrated that growing follicles greater than 10 mm in diameter ovulated in response to GnRH administration (Prescott *et al.*, 1992; Pursley *et al*., 1995; Silcox *et al.,* 1995; Martinez *et al*., 1999).

Wolfenson *et al*. (1994) studied the dynamics of follicular development by ultrasonography in cows following administration of a single dose of GnRH in the midluteal phase of the oestrus cycle. They opined that the preovulatory follicles following the injection of GnRH during the luteal phase were more homogenous and more oestrogen active.

McDougall *et al.* (1995) reported that administration of GnRH induced an LH surge with maximum LH concentration but with approximately half the duration, when compared to the endogenous LH release at the time of ovulation (Chenault *et al.,* 1975; Rahe *et al.,* 1980; Chenault *et al.*, 1990).

2.4.1 Response of GnRH at Different Stages of Oestrus Cycle in Post Partum Cows

A low ovulation rate following GnRH administration at the early stage of the oestrus cycle was related to the fact that proteins or mRNA for LH receptor were not expressed in the granulose cells of growing follicles during first 2 days of the follicular wave (Xu *et al.,* 1995; Bodensteiner *et al.,* 1996 Bao *et al.,* 1997) and follicles attained ovulatory capacity on day 5 to 9 of oestrus cycle leading to a high ovulation rate of 90 to 96 per cent. GnRH administration near midcycle resulted in a low ovuation rate of 54 per cent (Bodensteiner *et al.,* 1996; Bao *et al.,* 1997) and the ovulatory response in late oestrus cycle was basically dependent on the presence of a new follicular wave at the time of treatment (Vasconcelos *et al.,* 1997). In another study Vasconcelos *et al.* (1999) obtained 77 per cent ovulation rate in response to GnRH administration during late oestrus cycle where as Pursley *et al.* (1996) recorded 100 per cent ovulation rate.

Ginther *et al.* (1996) reported that during midcycle there was loss of functional dominance in most of the large follicles of the first follicular wave resulting in increased serum FSH concentrations and emergence of a new follicular wave. Further it was reported that the day of the oestrus cycle for loss of functional dominance was influenced by many factors like nutrition, heat stress and growth hormone treatment (Lucy *et al.,* 1992; Murphy *et al*; 1991; Wherman *et al.,* 1993; Kirby *et al.,* 1997).

2.5 GnRH ADMINISTRATION IN DAIRY COWS

Lee *et al*. (1983, 1985) reported that administration of GnRH at the time of breeding induced an additional surge of LH that enhanced luteinisation of granulose cells which ensured adequate production of progesterone to maintain pregnancy and resulted in enhanced pregnancy rate whereas Mee *et al*. (1990) observed that GnRH administration at first service postpartum failed to improve pregnancy rate, regardless of the timing of GnRH administration or AI relative to the onset of oestrus.

Rosenberg *et al*. (1991) reported that GnRH increased the fertility of cows when administered soon after the onset of oestrus. In cows those required more than one AI, GnRH improved fertility when AI was performed late in oestrus.

Pursley *et al*. (1995) obtained synchronized ovulation in dairy cows within 8 h using a sequence of GnRH + $PGF_2\alpha$ + GnRH (Ovsynch). This protocol allowed effective management in lactating dairy cows with difficulty of oestrus detection, and provided similar pregnancy rates when compared to AI at natural oestrum (Wolfenson *et al*. 1994; Pursley *et al*., 1997a and 1997b).

Administration of GnRH at the onset of oestrus decreased the time interval from the onset of oestrus to the LH surge peak but it did not affect the interval from the peak of LH surge to ovulation (Kaim *et al*., 2003).

Peters and Pursley (2003) opined that in order to control the time of LH surge and ovulation, the final GnRH administration of Ovsynch must be given prior to a spontaneous LH surge to control the time of AI.

2.5.1 Fertility After GnRH Treatment

Ryan *et al*. (1994) reported that treatment of lactating dairy cows with GnRH analogue either at AI or on day 12 after AI, altered endocrine responses and ovarian follicular population, but did not affect pregnancy rates.

High efficacy of oestrus synchronization was observed in normal cows when GnRH was administered 48 h after $PGF_2\alpha$ with good conception rate (Doleiel *et al*., 2002).

Administration of 10µg Buserelin just before insemination resulted in a conception rate of 20 per cent higher than control but GnRH was found to have no influence on subsequent serum progesterone level on 14^{th} and 22^{nd} day post insemination (Schelar *et al*., 2002).

2.6 GnRH- $PGF_2\alpha$ PROTOCOL IN DAIRY CATTLE

In lactating dairy cows synchronization of oestrus with $PGF_2\alpha$ protocols gave varying results because of the abnormal pattern of ovarian activity in postpartum dairy cows (Bulman and Wood, 1980; Stevenson and Pursley, 1994). This variation in time of oestrus response was related to the serum progesterone concentration and differences in the developmental stage of the preovulatory follicle at the time of $PGF_2\alpha$ administration (King *et al.,* 1982; Fortune *et al.*, 1991).

Several researchers described a higher rate of oestrus synchronization when GnRH was administered 6 or 7 days before $PGF_2\alpha$ compared with $PGF_2\alpha$ alone (Thatcher *et al.,* 1989; Twagiramungu *et al.,* 1992a; Stevenson *et al.,* 1999; Coyan *et al*., 2003).

A few reports regarding the simultaneous administration of GnRH and $PGF_2\alpha$ for oestrus synchronization with less promising results were recorded in dairy cows (Stevens *et al.,* 1993; Birnie *et al.,* 1997).

GnRH-$PGF_2\alpha$ regime failed to induce oestrus in some cows due to incomplete luteolysis after $PGF_2\alpha$ treatment (Twagiramungu *et al.,* 1994) or because of differences in pituitary LH release at the time of treatment (De Rensis *et al.,* 1999). In addition Stevenson *et al.* (1996) and LeBlanc *et al.* (1998) reported no advantage of adding GnRH on day 7 of a synchronization program based on double $PGF_2\alpha$ at 14 day interval.

2.7 GnRH - $PGF_2\alpha$ - GnRH COMBINATION

To synchronize ovulation within a short time and to enable TAI in the GnRH-$PGF_2\alpha$ regime an additional GnRH was included after $PGF_2\alpha$ administration (Youngquist and Braun, 1986; Plata *et al.,* 1990; Pursley *et al.,* 1995; Silcox *et al.,* 1995; Twagiramungu *et al.,* 1995a; Thatcher *et al.,* 1996; Peters *et al.,* 1999).

Addition of a second dose of GnRH given 48 h after $PGF_2\alpha$ improved the precision of oestrus over an 8 h period from 24 to 32 h after the second GnRH dose (Pursley *et al*., 1995). The success of this addition to the combined GnRH-$PGF_2\alpha$ regime in dairy cattle gave rise to Ovsynch or TAI protocol which allowed successful FTAI without the need for oestrus detection. Further this protocol could be effectively used to control time to first and subsequent inseminations by AI (Pursley *et al.,* 1995; Zeroual *et al.,* 1995; Pursley and Bellow, 2007). In addition, Geary *et al.* (1998) reported satisfactory ovulation rate with Ovsynch protocol in anoestrous cows.

Ovsynch protocol was slightly modified, so that the second dose of GnRH could be administered at 36 h instead of 48 h after $PGF_2\alpha$ (Pursley *et al.,* 1997a;

Nebel and Jobst, 1998). In another study Pursley *et al.* (1998) reported that AI performed close to 16 hours after the second dose of GnRH in the Ovsynch protocol was optimal to obtain satisfactory conception rate.

Fricke *et al.* (1998) and Yamada *et al.* (2002) reported that the reproductive performance of dairy cattle was unaffected when the GnRH dose was reduced to half.

2.7.1 Influence of the Stage of Oestrus Cycle at the Time of Initiation of Ovsynch Protocol

The success of Ovsynch program was proved to be influenced by the number of follicular waves (Pursley *et al.,* 1997b) as well as stage of the oestrus cycle when the first GnRH dose was administered (Vasconcelos *et al.,* 1997, 1999; Moreira *et al.,* 2000). The major limitation of Ovsynch was the wide variability in synchronization rate (Vasconcelos *et al.,* 1999). However Keister *et al.* (1999) reported better fertility in Ovsynch protocol initiated irrespective of the day of cycle.

Moreira *et al.* (2001) followed presynchronization in cows using two $PGF_2\alpha$ doses given 14 days apart to initiate the Ovsynch protocol at the targeted early luteal phase and obtained good conception rate.

2.7.2 Fertility Following Ovsynch in Cattle

Many researchers reported increased pregnancy rates in dairy cows subjected to Ovsynch protocol (Burke *et al.,* 1996; Pursley *et al.,* 1997b; Geary *et al.,* 1998; Mialot *et al.,* 1999; Cartmill *et al.,* 2001a). Burke *et al.* (1996) recommended TAI following Ovsynch protocol and Yamada *et al.* (1999) and Momicilovic *et al.* (1998) advocated Ovsynch for improving reproductive managemaent in dairy cows since it avoided oestrus detection.

In heifers, TAI following Ovsynch had no beneficial effects due to an inconsistent follicular wave pattern (Pursley *et al.,* 1997 b).

2.7.3 Interval Between First GnRH Dose and $PGF_2\alpha$ Administration

Increased time interval between first GnRH dose and $PGF_2\alpha$ in the Ovsnch protocol resulted in the development of a persistent dominant follicle or atresia of dominant follicle resulting in reduced fertilty (Savio *et al*., 1993; Stock and Fortune, 1993; Mihim *et al*., 1994; Revah and Butler, 1996). A seven day period was reported to be an appropriate interval between first GnRH and $PGF_2\alpha$ to allow for follicular development and CL maturity at the time of induction of luteolysis (Pursley *et al*., 1995).

2.7.4 Influence of Progesterone Level at the Time of $PGF_2\alpha$ Treatment

Pursley *et al*. (1995) observed that the pregnancy rates following Ovsynch treatment was similar in cows regardless of the progesterone level at the time of $PGF_2\alpha$ administration whereas heifers with low progesterone concentration at the time of $PGF_2\alpha$ injection had lower pregnancy rates. On the contrary, Burke *et al.* (1996) reported that conception rate following Ovsynch program was influenced positively by the plasma concentration of progesterone.

Reproductive performance of cows synchronized with Ovsynch protocol could be improved by supplementing progesterone during the period between GnRH and prostaglandin treatment (Roy and Twagiramugu, 1999; Xu and Burton, 2000; Xu *et al.,* 2000).

2.7.5 Influence of Milk Yield, Stage of Lactation and Body Condition Score on Fertility

Burke *et al*. (1996) and Mattos *et al.* (2001) found positive influence of body condition score on pregnancy rate following Ovsynch treatment whereas

Momicilovic *et al.* (1998) recorded no effect of body condition score and parity on pregnancy rates.

Pursley *et al*. (1997a) observed that pregnancy rates following Ovsynch in dairy cattle was found to be correlated with lactation stage and opined that Ovsynch protocol initiated 76 days after calving resulted in improved pregnancy rates over those initiated between 60 and 75 day postpartum.

Vasconcelos *et al.* (1999) observed that high milk production was positively correlated with increased follicular size leading to lowered fertility following Ovsynch. Several researchers opined that high milk production led to reduced serum progesterone and consequent increased LH pulse frequency which resulted in increased follicular size (Roberson *et al.,* 1989; Bergefelt *et al.,* 1991; Adams *et al.,* 1992).

2.8. SERUM PROGESTERONE LEVEL IN DAIRY COWS

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 level 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, increased from about day 5 of the cycle to reach the levels of 6.0 ng/ml to 10.0 ng/ml between day 10 and 16 and the level abruptly came down to 0.5 ng/ml just prior to the next oestrus.

Serum progesterone level gradually increased from 1ng/ml by day 4 to 2.8 to 6 ng/ml by day 8 to 10 of the cycle in dairy cows (Sartori *et al.,* 2002, 2004).

Materials and Methods

3. MATERIALS AND METHODS

The study on oestrus synchronization and TAI for improving reproductive efficiency in postpartum dairy cows was performed in crossbred animals belonging to University Livestock Farm, Mannuthy during the period from June 2007 to May 2008. The cows were maintained under ideal managemental conditions and the practice of weaning calves immediately after parturition was followed in the farm. Those animals having a body condition score of 2.5 to 3.5 (Edmonson *et al.,* 1989) were selected at random for the study.

Detailed clinico-gynaecological examination was conducted and thirty cows with normal parturition and puerperium were selected at random and allotted to five groups. Cows with clinical problems detected during the course of study such as mastitis, lameness and digestive disorders were not included in the study. Data regarding oestrus response, time taken for onset of oestrum, duration of oestrum, uterine tonicity, ovarian status and intensity of oestrum of different groups were collected.

3.1 THERAPEUTIC REGIME ADOPTED

Animals were randomly allotted to five groups of six each and subjected to following treatment regime.

Group I

Induction of oestrus and ovulation was done by administering GnRH analogue 20µg *(5ml Buserelin) intramuscularly on day 40 postpartum, followed by $PGF_2\alpha$ analogue ** (2 ml Cloprostenol) 500µg on day 7 intramuscularly.

*RECEPTAL, 10ml vial, buserelin acetate 0.0042 mg per ml, synthetic GnRH analogue, Intervet International GmbH.

**CLOSTENOL, 2ml vial, cloprostenol sodium 263 µg per ml, synthetic prostaglandin F_2 alpha analogue, Sarabai Zydus.

GnRH analogue was administered at a dose of 10µg (2.5 ml Buserelin) on day 9, followed by TAI at 24th and 32nd hours. Serum progesterone was estimated on day 0, 7, 9 and during induced oestrum.

Group II

Induction of oestrus and ovulation was done by administering GnRH analogue 20µg intramuscularly on day 40 postpartum, followed by $PGF_2\alpha$ analogue 500µg intramuscularly on day 11. GnRH analogue was administered at a dose of 10µg on day 13, followed by TAI at 24th and 32nd hours. Serum progesterone was estimated on day 0, 11, 13 and during induced oestrum.

Group III

Induction of oestrus was done by administering $PGF_2\alpha$ analogue 500µg intramuscularly on day 40 postpartum. A second dose of $PGF_2\alpha$ analogue at the same dose was administered on day 11, followed by TAI at 72nd and 80th hour. Serum progesterone was estimated on day 0, 11 and during induced oestrum.

Group IV

Induction of oestrus was done by administering $PGF_2\alpha$ analogue 500µg intramuscularly on day 40 postpartum in animals having a functional CL and double insemination at an interval of 8 h was done on observed oestrum. Serum progesterone was estimated on day 0 and during induced oestrum.

Group V

Cows showing first natural postpartum oestrus with pronounced oestrus signs formed the control group. Serum progesterone was estimated on day 40 postpartum and during observed oestrum.

Pregnancy diagnosis was done by rectal examination of genitalia at 60 days after AI in all the groups and the data were subjected to statistical analysis.

3.2 OESTRUS RESPONSE

In all the groups detailed clinico-gynaecological examination was conducted to evaluate changes in the tubular genitalia and ovaries.

3.2.1 Time Taken for Induction of Oestrus

All experimental animals were closely observed for the signs of oestrus after hormone administration and those found in oestrum were confirmed by rectal palpation of internal genitalia. The interval from the administration of $PGF_2\alpha$ to the onset of oestrum was recorded as the time taken for induction of oestrus.

3.2.2 Duration of Oestrus

Duration of oestrum was monitored by close observation of clinical signs like mucus discharge, vulval oedema, and hyperemia of vestibular mucous membrane, bellowing and mounting in all the groups. The period from the beginning to the end of exhibition of clinical signs was considered as the duration of oestrus.

3.2.3 Physical Changes in the Tubular Tract

Physical changes in the reproductive tract of all animals such as oedema of vulval lips, hyperemia of vestibular mucous membrane, and tonicity of uterine horns were recorded and graded as low, medium and high.

3.2.4 Intensity of Oestrus

Intensity of oestrus was graded as high, medium and low from clinical and behavioral manifestations.

3.2.5 Detection of Ovarian Status

Both the ovaries were palpated for the presence of follicular activity and corpus luteum.

3.3 ESTIMATION OF SERUM PROGESTERONE LEVEL

Blood samples were collected on days mentioned in the chart below by jugular venipuncture using 18 gauge hypodermic needle and syringe into clean sterilized glass tubes and allowed to clot.

Groups	Days of blood collection					
	0	7	9	11	13	Day of oestrus
Group I	✓	✓	✓			✓
Group II	✓			✓	✓	✓
Group III	✓			✓		✓
Group IV	✓					✓
Group V	✓					✓

Clear serum was separated and stored in separate serum cryovials and stored at -20°C refrigeration. Progesterone level in the serum was estimated by Radio Immuno Assay (RIA) using commercially available RIA progesterone kit (Immunotech Bechman Coulter, France) at Radio Tracer Laboratory, Kerala Agricultural University. A 100 tube kit for progesterone estimation contained 2 x 50 antiprogesterone antibody coated tubes, 55 ml vial containing185 KBq (kilobecquerels) of ^{125}I labeled progesterone with buffer and proteins, six 0.5 ml vials containing concentration of progesterone for a standard range from 0 to 60 ng/ml in human serum with sodium azide and one 0.5 ml vial containing control.

The RIA method followed in the present study was validated for use in cows by Guibault *et al.* (1988). The sensitivity of the assay was 0.05 ng/ml progesterone. The intra assay coefficients of variation were below or equal to 5.8 per cent for serum and inter assay coefficients of variation were below or equal to 9.0 per cent of serum. Measurement range was from 0.05 - 60 ng/ml.

3.3.1 Principle

The RIA progesterone procedure is a solid phase radioimmunoassay for the direct measurement of progesterone in serum or plasma wherein ^{125}I labeled progesterone competes with progesterone in the test sample for antibody sites for a fixed time. Because the antibody is immobilized to the wall of a polypropylene tube, simply decounting the supernatant suffices to terminate the competition and to isolate the antibody bound fraction of the radio labeled progesterone. Counting the bound radioactivity in the gamma counter yields count per minute (CPM) which can be converted by means of a calibration curve to a measure of progesterone present in the serum sample.

3.3.2 Procedure

All the components in the progesterone assay kit and serum samples were brought to room temperature. Four plain polypropylene tubes were labeled for total counts and nonspecific binding (NSB) in duplicate. Six progesterone antibody coated tubes A (maximum binding) and B through F were labeled for calibrators. Additionally, antibody coated tubes were labeled for controls and test samples. Initially 50 µl of the zero calibrator was pipetted into the NSB and A tube and 50 µl of each of the calibrators B through F was added into corresponding labeled tubes. To coated tubes labeled for control and test samples sequentially added 50 µl of control and samples. To every tube 500 µl of ^{125}I labeled progesterone was added. The samples were mixed well in a vortex mixer and incubated for one hour at room temperature with shaking at 350 rpm. The

contents of the tube were aspirated carefully excepting the 2 tubes for total CPM. The tubes were allowed to drain for three hours. The radioactivity was determined in the gamma counter.

3.3.3 Calculation

The average NSB coated CPM was first calculated for each pair of tubes. Net counts = Average CPM minus Average NSB CPM

The B/T per cent of each sample was calculated (Bound count per minute/ total count per minute x 100). Using the semi-logarithmic graph paper B/T per cent was plotted on the vertical axis against the progesterone concentration of the calibrators on the horizontal axis for each of the calibrators A through F. Then the curve was drawn approximating these points. Progesterone concentration in the serum samples were estimated from the curve by interpolation.

3.4 CONCEPTION RATE

Pregnancy was confirmed in all the groups, 60 days after AI by rectal examination of genitalia. Conception rate of all treatment groups were compared with that of control.

3.5 CALVING TO CONCEPTION INTERVAL

The interval from calving to conception in all the groups was calculated. In addition data regarding calving to conception interval of the herd during the period from April 2006 to March 2008 were collected for comparison.

3.6 TREATMENT COST

The treatment cost incurred for the purchase of hormones for the experimental groups were calculated.

3.7 STATISTICAL ANALYSIS

The data obtained were compiled and subjected to statistical analysis as per Snedecor and Cochran (1985).

Results

4. RESULTS

Results of the investigations on synchronization of ovulation and timed artificial insemination to improve fertility in postpartum dairy cows are presented in tables 1-5 and figures 1-10.

4.1 OESTRUS RESPONSE TO DIFFERENT SYNCHRONIZATION PROTOCOLS

Oestrus response after the administration of hormones in Group I to IV is presented in Table 1 and Fig.1. Out of six animals each in the treatment groups 5, 2, 4 and 6 numbers in group I to IV responded to treatment by exhibiting oestrus signs indicating an efficacy of 83.33, 33.33, 66.67 and 100 per cent respectively.

4.1.1 Time Taken for Induction of Oestrus

The time taken in hours for induction of oestrum in Group I to IV animals were 52.50 ± 0.99, 52.33 ± 0.71, 52.83 ± 1.40 and 53 ± 0.97 h respectively (Table 1 and Fig.2). Analysis of data revealed that there was no significant difference in the time taken for induction between four groups.

4.1.2 Duration of Oestrus

The duration of oestrus in Groups I to V was 37.33 ± 0.71, 35.67 ± 0.88, 40.50 ± 0.76, 38.83 ± 0.83 and 39.83 ± 0.48 h respectively (Table 1 and Fig. 2). Analysis of data revealed that there was no significant difference in the duration of oestrum between groups after induction.

4.1.3 Physical Changes in the Reproductive Tract During Oestrus

Physical changes in the reproductive tract of animals in the experimental and control group were graded as high, medium and low and presented in Table 2.

The percentage of animals showing high, medium and low degrees of vulval oedema respectively were 0, 66.67 and 16.67 in group I, 0, 33.33 and 0 in group II, 33.33, 33.33 and 0 in group III, 66.67, 16.67 and 16.67 in group IV and 66.67, 33.33 and 0 in group V (Table 2).

The corresponding data denoting hyperaemia of vestibular mucous membrane were 0, 33.33 and 50 in group I, 0, 16.67 and 16.67 in group II, 16.67, 50 and 0 in group III, 66.67, 16.67 and 16.67 in group IV and 50, 33.33 and 16.67 in group V respectively (Table 2).

The percentage of animals showing high, medium and low degrees of uterine tonicity were 0, 50 and 33.33 in group I, 0, 33.33 and 0 in group II, 33.33, 33.33 and 0 in group III, 83.33, 16.67 and 0 in group IV and 50, 50 and 0 in group V respectively (Table 2).

4.1.4 Intensity of Oestrus After Induction

Intensity of oestrus after induction were noted and classified as high, medium and low. The percentage of animals showing high, medium and low intensity of oestrum were 0, 33.33 and 50 in group I, 0, 33.33 and 0 in group II, 33.33, 33.33 and 0 in group III, 83.33, 16.67 and 0 in group IV, 83.33, 16.67 and 0 in group V respectively (Table 2 and Fig. 3).

4.2 DETECTION OF OVARIAN STATUS

Ovarian changes observed during induced oestrus in treated animals and during natural oestrus in control group were tabulated in Table 3.

4.2.1 Ovarian Status

In Group I out of six animals examined, five had palpable graafian follicle (GF) and six had regressing corpus luteum (RCL) in their ovaries. The corresponding data for Group II animals were two and three respectively. In Group III out of six animals examined, four animals each had palpable GF and RCL in their ovaries. The corresponding data for group IV animals were six each. In the control, all the six animals had palpable GF and RCL in their ovaries.

4.3 SERUM PROGESTERONE LEVEL ON DIFFERENT DAYS OF HORMONE ADMINISTRATION IN EXPERIMENTAL AND CONTROL GROUPS

On stratification of data based on conception in group I, the mean serum progesterone level on day 40, 47, 49 and 50 postpartum for those conceived were 1.56 ± 0.69, 5.00 ± 0.94, 0.55 ± 0.09 and 0.36 ± 0.06 ng/ml respectively. For those animals in group I that did not conceive the corresponding values for the same days were 0.36 ± 0.01, 0.76 ± 0.61, 0.40 ± 0.11 and 0.32 ± 0.08 ng/ml. In group II, the mean serum progesterone level on day 40, 51, 53 and 54 for the conceived were 0.49 ± 0.23, 1.59 ± 0.59, 0.35 ± 0.13 and 0.88 ± 0.13 ng/ml respectively and for those that did not conceive the corresponding values were 0.99 ± 0.44, 0.35 ± 0.20, 0.31 ± 0.07 and 0.88 ± 0.21 ng/ml. In group III, the serum progesterone level on day 40, 51 and 54 for the conceived were 0.83 ± 0.19, 3.05 ± 0.38 and 0.33 ± 0.10 ng/ml respectively and the corresponding values for those that did not conceive were 0.20 ± 0.05, 0.34 ± 0.11 and 1.39 ± 0.01 ng/ml respectively. In group IV, the conceived animals had mean serum progesterone level 2.77 ± 0.38 and 0.46 ± 0.12 ng/ml on day 40 and on observed oestrum respectively, while for the one that did not conceive the corresponding values were 1.00 and 0.50 ng/ml. In the control, those that conceived had mean serum progesterone level 1.16 ± 0.27 and 0.48 ± 0.03 ng/ml on day 40 and on observed oestrum respectively and for those animals that did not conceive the

corresponding values were 1.60 ± 0.85 and 0.69 ± 0.24 ng/ml (Table 4). The serum progesterone level in responded and non responded animals in each group and control were shown in Fig. 4-8.

Statistical analysis of the data revealed difference in progesterone concentration on day 40 between the groups and the fourth group differed significantly from Group I to III and control.

4.4 CONCEPTION RATE

The conception rate after induction in groups I to V were 66.67, 33.33, 66.67, 83.33 and 66.67 per cent respectively. The overall conception rate in groups I to V were 83.33, 83.33, 66.67, 83.33 and 66.67 per cent respectively (Table 5 and Fig. 9).

4.5 CALVING TO CONCEPTION INTERVAL

The interval from calving to conception was 54.2 ± 4.2, 64.2 ± 4.16, 54 and 43 days in Group I to IV respectively. The mean calving to conception interval in the experimental groups was 53.84 ± 2.31 days where as in the control it was 95 ± 6.19 days. The interval from calving to conception for the herd during the period from 2006 April to 2008 March was 200.78 ± 15.97 days (Table 5 and Fig. 10).

4.6 TREATMENT COST

The treatment cost incurred for the purchase of hormones for individual cows of Group I to IV were Rs.422, Rs.422, Rs.250 and Rs.125 respectively.

Table 1. Oestrus response in experimental and control groups

Groups	Group I GnRH (0) - PG (7) - GnRH (9) - DAI (10) n = 6	Group II GnRH (0) - PG (11) - GnRH (13) - DAI (14) n = 6	Group III PG (0) - PG (11) - DAI (14) n = 6	Group IV PG (0) DAI n = 6	Group V Control n = 6
No: of animals responded to oestrus induction	5	2	4	6	-
Percentage of animals responded to oestrus induction	83.33	33.33	66.67	100	-
Time taken for induction of oestrum (Mean ± SE hours)	52.50 ± 0.99	52.33 ± 0.71	52.83 ± 1.40	53 ± 0.97	-
Duration of oestrum (Mean ± SE hours)	37.33 ± 0.71	35.67 ± 0.88	40.50 ± 0.76	38.83 ± 0.83	39.83 ± 0.48

Table 2. Intensity of oestrum and changes in the genital tract in experimental and control groups

Groups	Group I			Group II			Group III			Group IV			Group V		
Degree of intensity	High	Medium	Low	High	Medium	Low	High	Medium	Low	High	Medium	Low	High	Medium	Low
Vulval oedema Number	-	4	1	-	2	-	2	2	-	4	1	1	4	2	-
Per cent	-	66.67	16.67	-	33.33	-	33.33	33.33	-	66.67	16.67	16.67	66.67	33.33	-
Hyperaemia Vestibular mucosa Number	-	2	3	-	1	1	1	3	-	4	1	1	3	2	1
Per cent	-	33.33	50	-	16.67	16.67	16.67	50	-	66.67	16.67	16.67	50	33.33	16.67
Tonicity of uterus Number	-	3	2	-	2	-	2	2	-	5	1	-	3	3	-
Per cent	-	50	33.33	-	33.33	-	33.33	33.33	-	83.33	16.67	-	50	50	-
Intensity of oestrum Number	-	2	3	-	2	-	2	2	-	5	1	-	5	1	-
Per cent	-	33.33	50	-	33.33	-	33.33	33.33	-	83.33	16.67	-	83.33	16.67	-

Table 3. Ovarian changes in experimental and control groups on the day of oestrus

Groups	Right ovary			Left ovary			No palpable structures in either ovaries
	GF	RCL	GF + RCL	GF	RCL	GF + RCL	
Group I	1	2	2	1	1	1	-
Group II	1	1	1	-	1	-	3
Group III	2	2	1	1	1	-	2
Group IV	2	1	2	1	2	1	-
Group V	3	2	1	1	2	1	-

Table 4. Serum progesterone concentration on different days of hormone administration in experimental and control groups

Groups		Progesterone concentration (Mean ± SE ng/ml)							
		Day 0	Day 7	Day 9	Day 10	Day 11	Day 13	Day 14	Observed Oestrum
Group I	Conceived	1.56 ± 0.69	5.00 ± 0.94	0.55 ± 0.09	0.36 ± 0.06				
	Non-conceived	0.36 ± 0.01	0.76 ± 0.61	0.39 ± 0.11	0.32 ± 0.08				
Group II	Conceived	0.49 ± 0.23				1.59 ± 0.59	0.35 ± 0.13	0.88 ± 0.13	
	Non-conceived	0.99 ± 0.44				0.35 ± 0.20	0.31 ± 0.07	0.88 ± 0.21	
Group III	Conceived	0.83 ± 0.19				3.05 ± 0.38		0.33± 0.10	
	Non-conceived	0.19 ± 0.05				0.34 ± 0.11		1.39 ± 0.01	
Group IV	Conceived	2.77 ± 0.38							0.46 ± 0.12
	Non-conceived	1 ± 0							0.5 ± 0
Group V Control	Conceived	1.16 ± 0.27							0.48 ± 0.03
	Non-conceived	1.6 ± 0.85							0.69 ± 0.24

Table 5. Conception rate and calving to conception interval in experimental and control groups

Groups	No. of animals	No. conceived	Conception rate (per cent)	Overall conception rate (per cent)	Calving to conception interval (Mean ± SE days)
Group I	6	4	66.67	83.33	54.2 ± 4.2
Group II	6	2	33.33	83.33	64.2 ± 4.16
Group III	6	4	66.67	66.67	54
Group IV	6	5	83.33	83.33	43
Group V	6	4	66.67	66.67	95 ± 6.19
Experimental animals that conceived	19	-	-	-	53.84 ± 2.31
Herd	60	-	-	-	200.78 ± 15.97

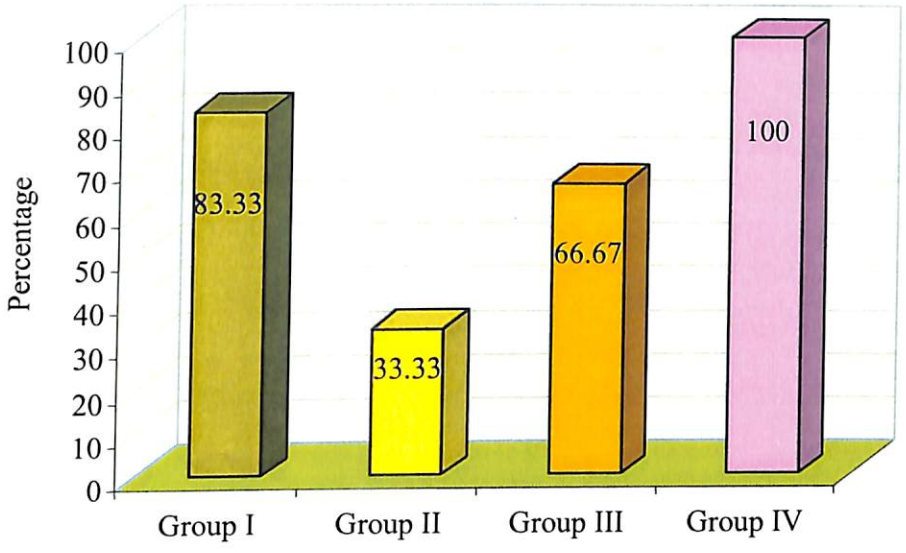

Fig. 1. Response to oestrus synchronization in experimental groups

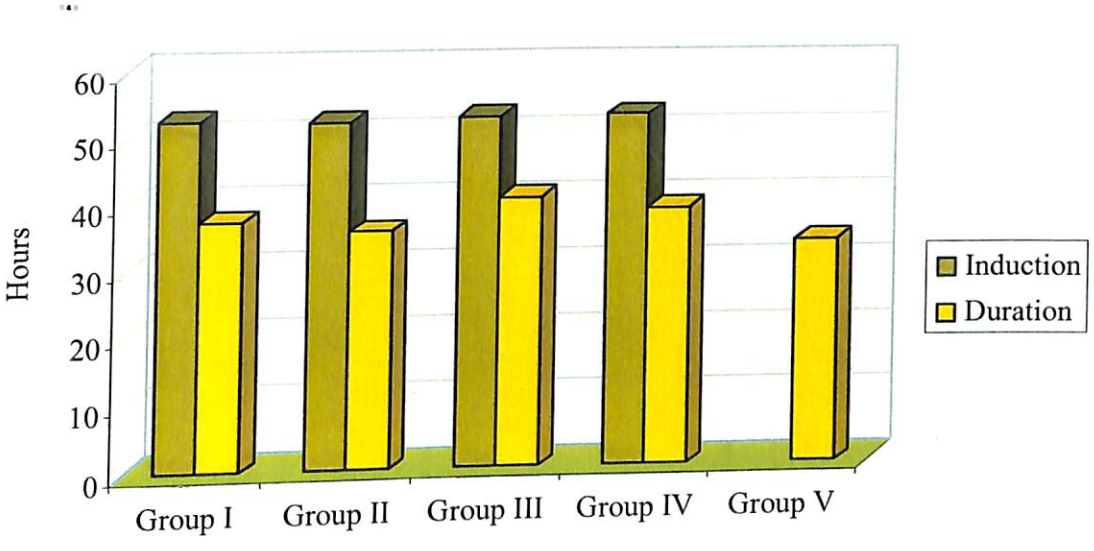

Fig. 2. Time taken for induction and duration of oestrus in experimental and control groups

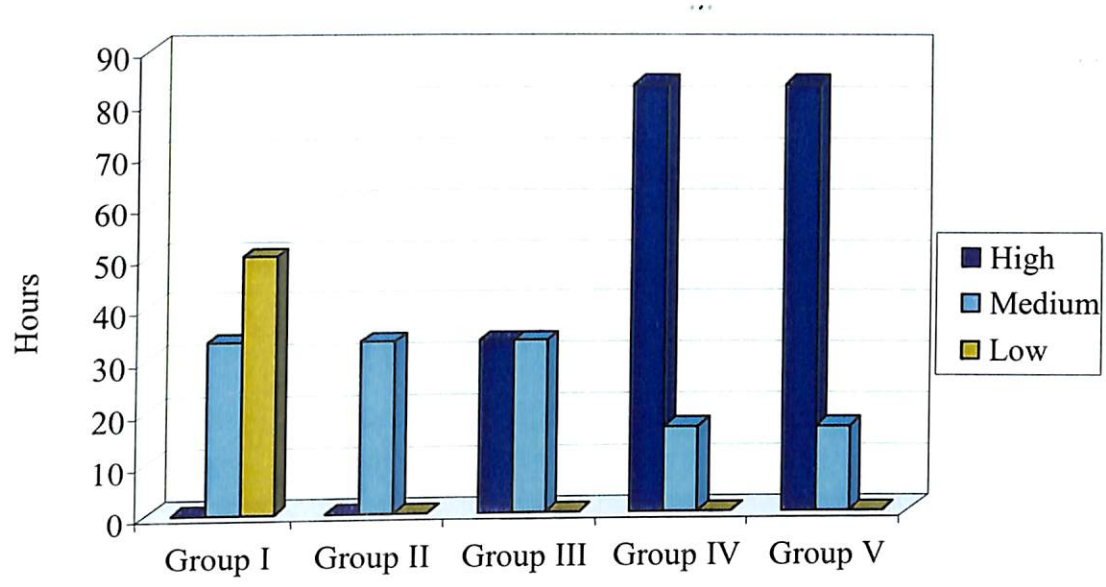

Fig. 3. Intensity of oestrus in experimental and control groups

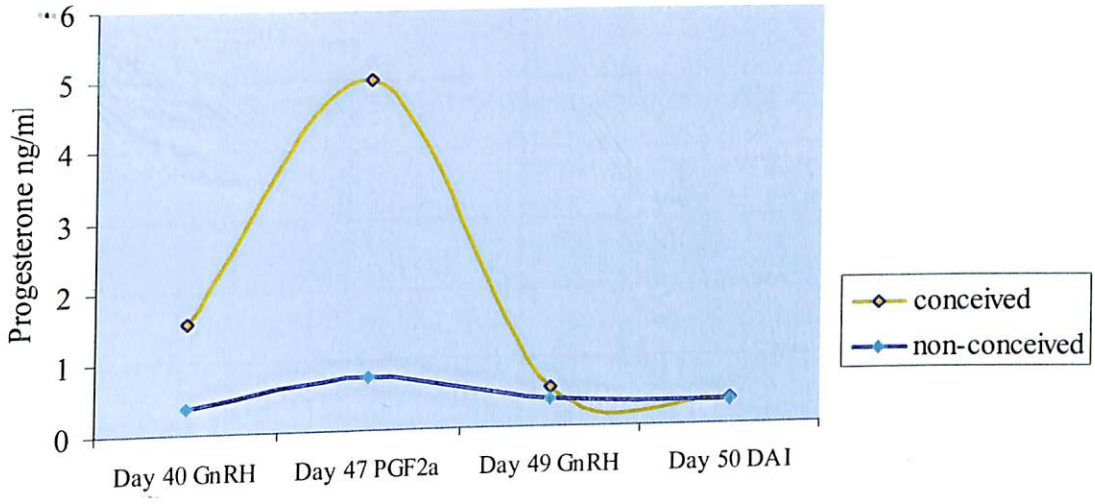

Fig. 4. Serum progesterone level on different days of hormone treatment in conceived and non-conceived animals of Group I

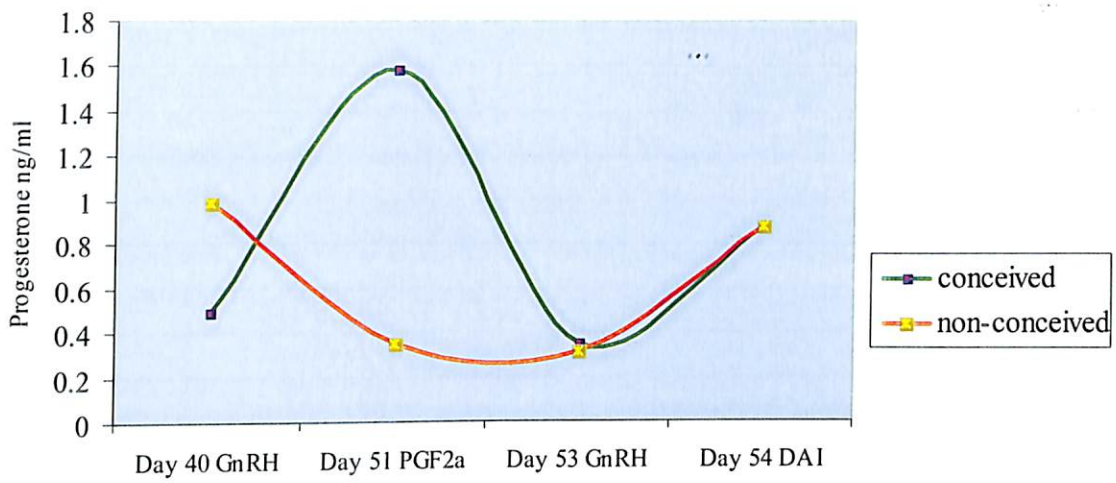

Fig. 5. Serum progesterone level on different days of hormone treatment in conceived and non-conceived animals of Group II

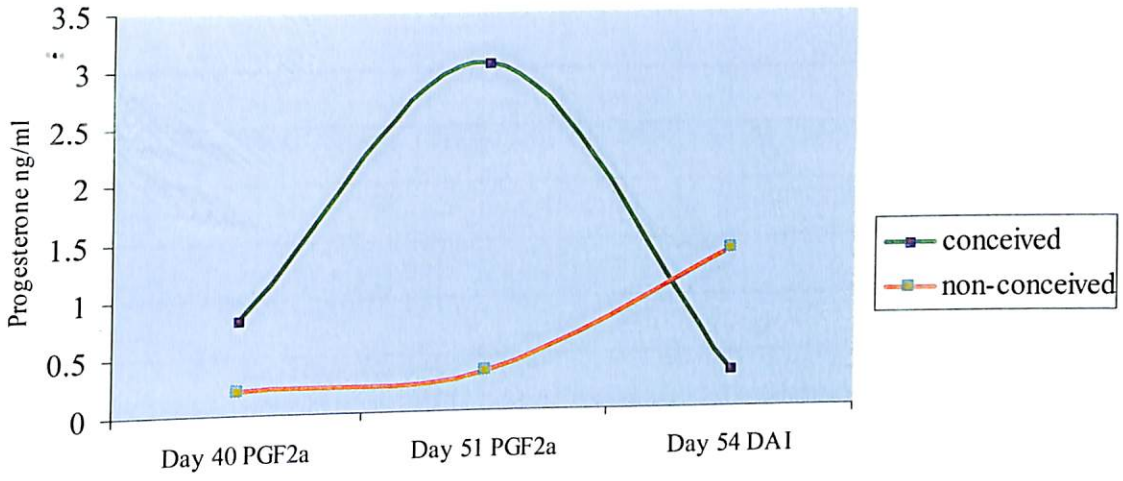

Fig. 6. Serum progesterone level on different days of hormone treatment in conceived and non-conceived animals of Group III

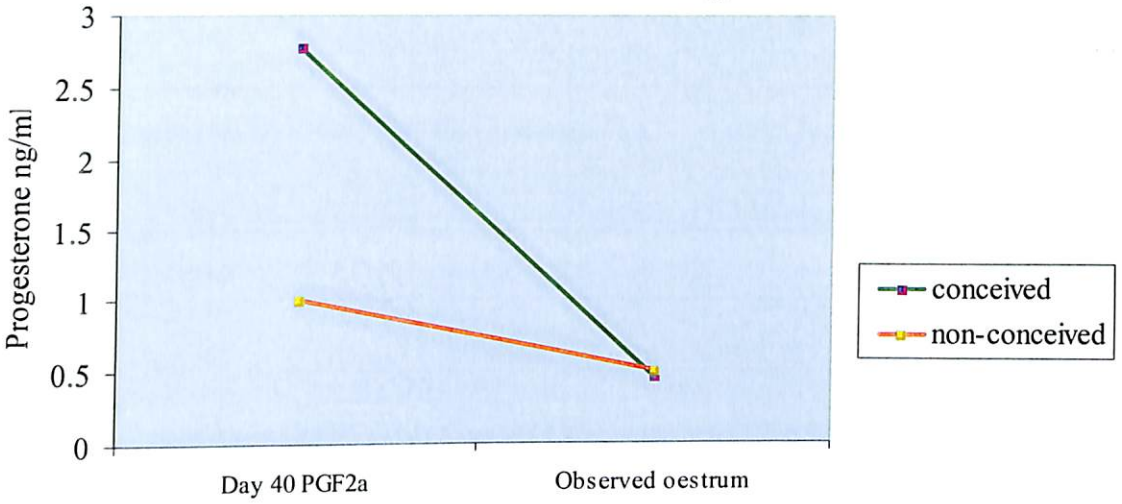

Fig. 7. serum progesterone level on different days in conceived and non-conceived animals of Group IV

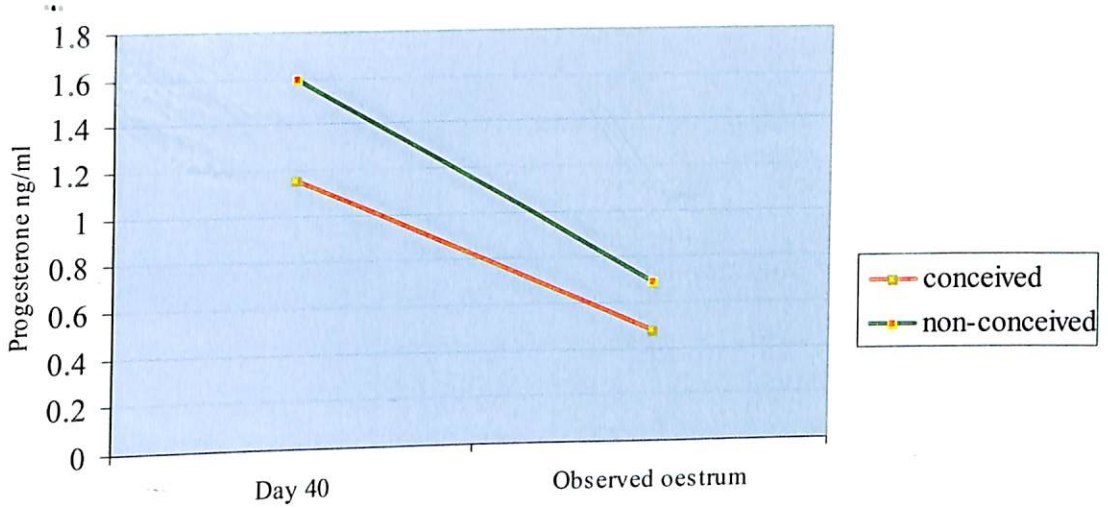

Fig. 8. Serum progesterone level on different days in conceived and non-conceived animals of control group

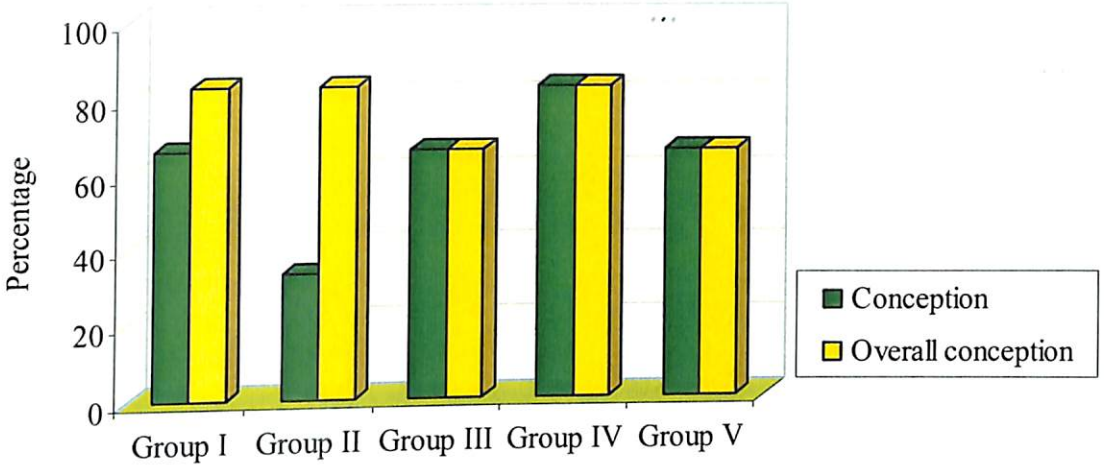

Fig. 9. Conception rate and overall conception rate in experimental and control groups

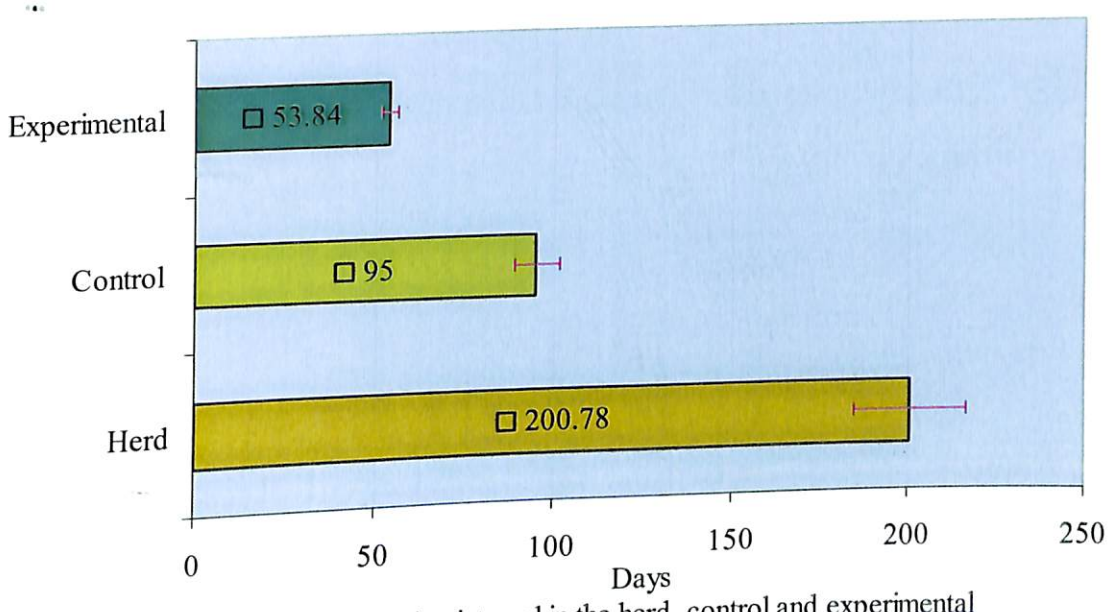

Fig.10. Calving to conception interval in the herd, control and experimental animals

Discussion

5. DISCUSSION

The present research work was undertaken to evaluate different oestrus and ovulation synchronization protocols and to recommend a better and more consistent TAI protocol for improving fertility and reducing intercalving interval in postpartum dairy cows.

5.1 OESTRUS RESPONSE TO DIFFERENT SYNCHRONIZATION PROTOCOLS

Perusal of data in Table 1and Fig.1 shows that 83.33 per cent in Group I, 33.33 per cent in Group II, 66.67 per cent in Group III and 100 per cent in Group IV responded to oestrus induction.

The oestrus response was varying in different groups which indicated that those animals which did not respond to synchronization were not having a functionally responsive mature CL at the time of $PGF_2\alpha$ administration. According to available published literatures, the oestrus response to $PGF_2\alpha$ administration in cows can vary from 54 to 95 per cent (Seguin *et al.,* 1983; Landivar *et al.,* 1985; Folman *et al*., 1990; Rosenberg *et al*., 1990; Kristula *et al.,* 1992).

5.2 TIME TAKEN FOR INDUCTION OF OESTRUM

In the present study the time taken for induction of oestrus after synchronization were 52.50 ± 0.99, 52.33 ± 0.71, 52.83 ± 1.40 and 53 ± 0.97 h in Group I to IV respectively which was similar to the findings of Ferguson and Galligan (1993) and Jeba (2005). Analysis of the data revealed no significant difference between the groups.

Ajitkumar (1994) and Senthilkumar and Rajasekhar (1998) reported that the time taken for induction of oestrus was over 63 h which was much higher compared to the findings in the present study. The variation in the time taken for oestrus onset observed by various researchers might be due to varying stages of oestrus cycle at the time of $PGF_2\alpha$ administration (King *et al*., 1982; Tanabe and Hann, 1984); varying stages of follicular wave development at the time of $PGF_2\alpha$ administration (Kastelic *et al*., 1990; Twagiramungu *et al*., 1992b; Ferguson and Galligan, 1993; Adams, 1994; Twagiramungu *et al*., 1995a; Diskin *et al*., 2002); differences in the developmental stage of preovulatory follicle (Fortune *et al*., 1991); might be related to the rate of progesterone decrease to the basal level or probably due to the use of various analogues of prostaglandin (Schams and Karg, 1982).

The time taken for onset of oestrum in Group I and II where GnRH was given to synchronize the recruitment of a new follicular wave, did not differ significantly from that of Group III and IV. Supporting the present study Twagiramungu *et al*. (1995a) reported that GnRH induced CL as well as spontaneous CL did not differ significantly in their responsiveness to prostaglandin induced luteolysis.

5.3 DURATION OF OESTRUM

The mean duration of oestrum in Group I to V were 37.33 ± 0.71, 35.67 ± 0.88, 40.50 ± 0.76, 38.83 ± 0.83 and 39.83 ± 0.48 h respectively. A comparatively lower duration of oestrus was observed in Group I and II. In these animals, exogenous administration of GnRH amplified the spontaneous endogenous preovulatory LH surge which might have hastened follicular maturation and ovulation. The finding in the present study agrees with that of Rosenberg *et al.* (1991).

Retrospective information generated from records made between 1991 and 1994 revealed that the mean duration of oestrus in cows maintained in tropical areas was 10.6 ± 4.5 h (Negussie *et al.,* 2002). The duration of oestrum in cows maintained in temperate zones ranged between 18 and 19 hours (Hafez and Hafez, 2001). In the present study, the duration of oestrum observed in experimental as well as control was much higher probably due to close monitoring of experimental animals for the presence of oestrus signs. Forster *et al.* (2007) opined that oestrus behaviour is not necessarily the best marker to predict the time of ovulation due to variation in the length of oestrus period supporting the findings in the present study. Hence TAI protocol followed in the experimental groups was very useful and can be recommended for closely confined animals.

5.4 PHYSICAL CHANGES IN THE REPRODUCTIVE TRACT

In the present study the physical changes in the reproductive tract was found to be more pronounced in the control group than in oestrus induced animals which was in agreement with the findings of Jacob (1993) and Jeba (2005). On the contrary Ajitkumar (1994) observed a marginal increase in physical changes of the reproductive tract in $PGF_2\alpha$ induced oestrus when compared to natural oestrus. Similarly GnRH administration did not influence the physical changes in the reproductive tract in treated animals.

5.5 INTENSITY OF INDUCED OESTRUM

The percentage of animals showing high, medium and low intensity of oestrum were 0, 33.33 and 50 in group I, 0, 33.33 and 0 in group II, 33.33, 33.33 and 0 in group III, 83.33, 16.67 and 0 in group IV, 83.33,16.67 and 0 in group V respectively. In the present study more numbers of animals showed higher intensity of oestrum in Group IV and Control. A lower intensity of oestrus observed in Group I and II might be due to artificially induced LH surge by the administration of GnRH which hastened early maturation and ovulation of

follicles resulting in cessation of oestrum. This finding agrees with that of Pursley *et al.* (1995).

5.6 ANALYSIS OF SERUM PROGESTERONE LEVEL

Statistical analysis of data regarding serum progesterone concentration on day 0 revealed difference in progesterone concentration between the groups and the fourth group differed significantly from Group I to III and V.

The mean serum progesterone level on day 0, 7, 9 and during induced oestrum in the conceived animals of Group I were 1.56 ± 0.69, 5.00 ± 0.94, 0.55 ± 0.09 and 0.36 ± 0.06 ng/ml respectively, confirming that the cows were in the early to mid luteal phase on day 0 (Edqvist *et al*., 1974; Sartori, *et al.,* 2002, 2004). Administration of GnRH caused an alteration of follicular distribution in the ovary by increasing the number of medium sized follicles and decreasing the number of large follicles by inducing luteinisation, atresia or formation of a secondary CL following ovulation as indicated by the higher serum progesterone level on day 7 (McNatty *et al.,* 1981; Thatcher *et al.,* 1989; Guilbault *et al*., 1990; Stevenson *et al.,* 1993). Subsequent administration of $PGF_2\alpha$ in Group I induced regression of the original or GnRH induced CL (Harrison *et al.*, 1985) and allowed final maturation of the synchronized dominant follicle (Schmitt *et al.,* 1996). This was confirmed by the serum progesterone levels on day 9 and during observed oestrum which was in agreement with the findings of Wishart *et al.* (1975) and Sartori *et al.* (2004).

The mean serum progesterone level on day 0, 7, 9 and during induced oestrum in Group I animals which did not conceive were 0.36 ± 0.01, 0.76 ± 0.61, 0.40 ± 0.11 and 0.32 ± 0.08 ng/ml respectively. Serum progesterone level confirmed that the cows were having less luteal activity on day 0. Serum progesterone level on day 7 confirmed that luteinisation did not occur in response to first GnRH. This might be due to the absence of GnRH sensitive follicles on

day 0 or because of differences in pituitary LH release at the time of treatment which agrees with the findings of various researchers (Xu *et al.,* 1995; Bodensteiner *et al.,* 1996; Bao *et al.,* 1997; De Rensis *et al.,* 1999; Vasconcelos *et al.,* 1999; Murugavel, 2003). From the findings it could be inferred that the non responded animals were lacking a functional CL at the time of $PGF_2\alpha$ administration and hence they did not respond to synchronization. These findings agree with that of Seguin *et al.* (1983), Landivar *et al.* (1985) and Kristula *et al.* (1992).

The mean serum progesterone level on day 0, 11, 13 and during induced oestrum in the conceived animals of Group II were 0.49 ± 0.23, 1.59 ± 0.59, 0.35 ± 0.13 and 0.88 ± 0.13 ng/ml respectively. Majority of animals in Group II did not respond to the protocol and the serum progesterone level in the non pregnant animals were 0.99 ± 0.44, 0.35 ± 0.20, 0.31 ± 0.07 and 0.88 ± 0.21 ng/ml respectively. In these animals the CL might have undergone premature regression or spontaneous luteolysis between the administration of first dose of GnRH and PGF2α, as indicated by serum progesterone level on day 11.The findings were in agreement with that of Vasconcelos *et al.* (1997, 1999) and Moreira *et al.* (2000).

The mean serum progesterone level on day 0, 11 and during induced oestrum in the conceived animals of Group III were 0.83 ± 0.19, 3.05 ± 0.38 and 0.33 ± 0.10 ng/ml respectively. These animals had very low serum progesterone level at the time of first $PGF_2\alpha$ administration but the progesterone level has increased significantly during the second $PGF_2\alpha$ administration. This resulted in most animals exhibiting oestrum after the second injection of $PGF_2\alpha$. On the day of second $PGF_2\alpha$ administration, a functional CL could be palpated in these animals agreeing with the findings of Larson and Ball (1992). The average serum progesterone level on day 0, 11 and during induced oestrum in the non pregnant animals of Group III were 0.20 ± 0.05, 0.34 ± 0.11 and 1.39 ± 0.01 ng/ml respectively, indicating that on both days of $PGF_2\alpha$ administration functional CL was absent and hence the animals failed to respond. The findings were in

agreement with that of Seguin *et al.* (1983), Landivar *et al.* (1985) and Kristula *et al***.** (1992).

The mean serum progesterone level on day 0 and during induced oestrum in conceived animals of Group IV were 2.77 ± 0.38 and 0.46 ± 0.12 ng/ml respectively, indicating the presence of a functional CL on day 0 which had responded to $PGF_2\alpha$ administration positively. The present study agrees with that of Folman *et al*. (1990) who observed that the level of progesterone prior to ovulation following the administration of prostaglandin affected the fertility of cows in synchronized oestrus. In a single animal of Group IV which failed to conceive, the serum progesterone level on day 0 and during induced oestrum were 1.00 and 0.50 ng/ml respectively. The serum progesterone level on day 0 was not adequate to bring an ovulatory response.

The mean serum progesterone level on day 40 postpartum and during observed oestrum in conceived animals of control group were 1.30 ± 0.29 and 0.55 ± 0.08 ng/ml respectively. The corresponding values for the nonconceived were 1.0 and 0.5 ng/ml. These animals exhibited natural oestrum at 95 ± 6.19 days post partum. This calls for intervention of reproductive cycle using exogenous hormones during early postpartum period.

5.7 CONCEPTION RATE

The conception rate in different oestrus synchronization protocol in Group I to V were 66.67, 33.33, 66.67, 83.33 and 66.67 per cent respectively and the overall conception rate in groups I to V were 83.33, 83.33, 66.67, 83.33 and 66.67 per cent respectively.

In Group I in which Ovsynch protocol was followed the pregnancy rate was 66.67 per cent during induced oestrus which was similar to that of control. However a higher overall conception rate of 83.37 per cent was obtained in the

treated animals. The result obtained in the present study was in agreement with earlier reports (Burke *et al.,* 1996; Pursley *et al.,* 1997b; Geary *et al.,* 1998; Momicilovic *et al.*, 1998; Mialot *et al.,* 1999; Yamada *et al.,* 1999; Cartmill *et al.,* 2001b).

In Group II in which Ovsynch protocol with variation in the interval between first GnRH and $PGF_2\alpha$ was tried, the conception rate was 33.33 with an overall conception rate of 83.33 per cent. Supporting the above findings Pursley and Bellow (2007) reported that increasing the time from first GnRH dose to $PGF_2\alpha$ in the Ovsnch protocol may allow a greater number dominant follicle to reach atresia and may result in a new follicular wave and the absence of a dominant follicle at the final GnRH dose resulting in ovulation 2 to 4 days after the final GnRH dose. As a second possibility they opined that extending the time may increase the age of the dominant follicle resulting in a persistent type dominant follicle at the time of administration of $PGF_2\alpha$ and final GnRH. When the persistent dominant follicle was allowed to ovulate, fertility was decreased when compared with that of younger ovulatory follicles (Savio *et al*., 1993; Stock and Fortune, 1993; Mihim *et al*., 1994; Revah and Butler, 1996).

An enhanced overall conception rate was obtained in Group I and II due to early initiation of ovarian cyclicity by the administration of exogenous GnRH.

Conception rate was highest in Group IV where $PGF_2\alpha$ was administered on confirmation of functional CL and AI done at observed oestrum. In this group $PGF_2\alpha$ administration induced lysis of CL and initiation of a new follicular wave resulting in ovulation of a healthy dominant follicle. The result obtained in the present study agrees with the findings of various researchers (Stevenson *et al.,* 1987; Wenzel, 1991; Archbald *et al.,* 1992; Murugavel *et al.,* 2003). Few researchers even reported above normal fertility following synchronization of oestrus with $PGF_2\alpha$ in cows (Deutsher *et al.,* 1982; McIntosh *et al.,* 1984; Plunket *et al.,* 1984; Lucy *et al.,* 1986; Leeba, 2003).

In Group III in which double $PGF_2\alpha$ regime was followed significant improvement in conception rate was not observed. Contradictory to the observations in the present study, Heuweiser *et al.* (1997) reported that reproductive performance in dairy cattle was improved following double $PGF_2\alpha$ treatment with out assessing ovarian status when compared with a single dose based on detecting a CL by rectal palpation or milk progesterone by RIA. However in the present study, no improvement in the overall conception rate was observed in single and double $PGF_2\alpha$ protocol as reported by Strelow (1993).

5.8 CALVING TO CONCEPTION INTERVAL

The mean calving to conception interval for the experimental animals, control and the herd were 53.84 ± 2.31, 95 ± 6.19 and 200.78 ± 15.97 days respectively. There was significant difference in the calving to conception interval between experimental group, control group and the herd. The calving to conception interval of control animals was significantly lower than the herd. The control animals were under close observation whereas this was not followed in the herd.

5.9 TREATMENT COST

The treatment cost involved is meager when compared to the loss incurred due to increased number of days open and cost incurred for replacement stock. Many reports suggest that reduced inter calving period in oestrus synchronized animals was due to early initiation of post partum ovarian cyclicity (Stevenson, 2001).

The findings in the present study suggest the need for detection of the ovarian status of early post partum cows before commencing any timed insemination protocol. The reproductive status of the herd could be assessed by skillful clinico-gynaecological examination together with estimation of serum/

plasma progesterone or milk progesterone. Assessing the ovarian status before applying a protocol of oestrus induction and TAI with good quality semen will improve the reproductive performance associated with a systematic breeding programme. Hence the study recommends stepwise assisted reproductive tactices involving assessment of reproductive status, augmenting early involution of uterus and initiating postpartum ovarian cyclicity to achieve the basic goal of reducing the intercalving interval. Prostaglandin therapy alone and its combination with GnRH at appropriate time were found to be helpful for attaining this goal.

Summary

6. SUMMARY

The present research work was undertaken to evaluate different oestrus and ovulation synchronization protocols and to recommend a better and more consistent timed artificial insemination (TAI) protocol for improving fertility and reducing intercalving interval in postpartum dairy cows. The study was performed in crossbred cows belonging to University Livestock farm, Mannuthy during the period from June 2007 to May 2008. Detailed clinico-gynaecological examination was conducted and thirty cows with normal parturition and puerperium were selected for the study and they were allotted at random to five groups of six each. Reproductive status of tubular genitalia and ovaries before, during and after treatment and intensity, duration and time taken for onset of oestrus in response to various synchronization protocols and during natural oestrus were studied in detail. Serum progesterone level before, during and after treatment were assayed. Efficacy of various synchronization protocols for inducing oestrus and ovulation were determined. Pregnancy diagnosis was done by rectal examination on day 60 after AI to determine the conception rate. Calving to conception interval for various synchronization protocols was calculated.

In Group I, 20µg of GnRH analogue (Buserelin) was administered intramuscularly on day 40 postpartum followed by 500µg of $PGF_2\alpha$ analogue (Cloprostenol) on day 7 intramuscularly and a second dose of 10µg GnRH was administered on day 9 followed by TAI at 24^{th} and 32^{nd} hours. In Group II, 20µg of GnRH analogue was administered intramuscularly on day 40 postpartum followed by 500µg of $PGF_2\alpha$ analogue intramuscularly on day 11 and a second dose of 10µg GnRH analogue was administered on day 13 followed by TAI at 24^{th} and 32^{nd} hours. In Group III, $PGF_2\alpha$ analogue 500µg was administered intramuscularly on day 40 postpartum followed by another same dose of $PGF_2\alpha$ on day11 and TAI at 72^{nd} and 80^{th} hour. In Group IV, cows with a palpable functional CL on day 40 postpartum were administered 500µg $PGF_2\alpha$ analogue and were inseminated at observed oestrus. Cows inseminated during first natural

post partum oestrus formed the control (Group V). In the experimental and control groups serum progesterone was estimated on day 40 postpartum, on days of hormone administration and during oestrum. Pregnancy diagnosis was done by per rectal palpation of genitalia at 60 days after AI in all the groups and the data were subjected to statistical analysis.

Response to oestrus synchronization was 83.33, 33.33, 66.67 and 100 per cent in Group I to IV respectively. In Group II in which Ovsynch protocol with $PGF_2\alpha$ administration on day 11 resulted in a lowered oestrus response compared to Group I Ovsynch. In double $PGF_2\alpha$ protocol a lowered oestrus response was obtained compared to single $PGF_2\alpha$ protocol in which the drug was administered to animals having a functional corpus luteum. The reason attributed was the presence of a functional CL in these animals on the day of $PGF_2\alpha$ administration.

The time taken for induction of oestrum was 52.50 ± 0.99, 52.33 ± 0.71, 52.83 ± 1.40 and 53 ± 0.97 h respectively in Group I to IV, but there was no significant difference between the groups. The duration of oestrus in groups I to V were 37.33 ± 0.71, 35.67 ± 0.88, 40.50 ± 0.76, 38.83 ± 0.83 and 39.83 ± 0.48 h respectively. In Group I and II, the duration of oestrum was comparatively lower than the other groups, which was due to the administration of GnRH.

The mean serum progesterone level on day 0, 7, 9 and observed oestrum for the conceived animals in Group I were 1.56 ± 0.69, 5.00 ± 0.94, 0.55 ± 0.09 and 0.36 ± 0.06 ng/ml. For those animals in Group I that did not conceive, the corresponding values for the same days were 0.36 ± 0.01, 0.76 ± 0.61, 0.40 ± 0.11 and 0.32 ± 0.08 ng/ml respectively. In Group II, the mean serum progesterone level on day 0, 11, 13 and observed oestrum for the conceived were 0.49 ± 0.23, 1.59 ± 0.59, 0.35 ± 0.13 and 0.88 ± 0.13 ng/ml respectively and those that did not conceive had mean serum progesterone level 0.99 ± 0.44, 0.35 ± 0.20, 0.31 ± 0.07 and 0.88 ± 0.21 ng/ml respectively. In Group III, the mean serum progesterone level on day 0, 11 and observed oestrum for the conceived were 0.83 ± 0.19, 3.05

± 0.38 and 0.33 ± 0.10 ng/ml respectively and for the non conceived the corresponding values were 0.20 ± 0.05, 0.34 ± 0.11 and 1.39 ± 0.01 ng/ml respectively. In group IV, the conceived animals had mean serum progesterone level 2.77 ± 0.38 and 0.46 ± 0.12 ng/ml respectively on day 0 and observed oestrum, while the corresponding values for the non conceived were 1.0 and 0.5 ng/ml respectively. In the control group those that conceived had serum progesterone level 1.30 ± 0.29 and 0.55 ± 0.08 ng/ml on day 40 and on observed oestrum and the corresponding values for the non conceived were 1.6 ± 0.85, 0.685 ± 0.235 ng/ml respectively. The results indicate that adopting a specific synchronization protocol applied according to the ovarian status rather than applying a single protocol regardless of the ovarian status can improve reproductive performance in post partum dairy cows.

The conception rates after synchronization in groups I to IV were 66.67, 33.33, 66.67, and 83.33 per cent respectively. The conception rate for the control was 66.67 per cent. The overall conception rate in groups I to V were 83.33, 83.33, 66.67, 83.33 and 66.67 per cent respectively.

In Group I and II, in which $PGF_2\alpha$ analogue was administered at varying intervals a conception rate of 66.67, 33.33 and overall conception rate of 83.33 per cent each was obtained. In Group III and IV, in which double and single $PGF_2\alpha$ protocol was followed a conception rate of 66.67 and 83.33 and an overall conception rate of 66.67 and 83.33 per cent was obtained. From the study, it could be confirmed that the higher conception rate and overall conception rate obtained in Ovsynch protocol was due to triggering of the neuroendocrine mechanism by GnRH. The second GnRH administration might have resulted in greater ovulatory response and augmentation of luteal activity. The highest conception rate was obtained in single regime $PGF_2\alpha$ treated animals compared to double regime since the animals in single regime was selected based on the presence of functional CL in the ovaries. This was later confirmed by the estimation of serum progesterone level.

The interval from calving to conception was 54.2 ± 4.2, 64.2 ± 4.16, 54 and 43 days in Group I to IV respectively, where as in the control group it was 95 ± 6.19 days. The mean calving to conception interval in the treated groups was 53.84 ± 2.31 days which was significantly lower than the control. The mean calving to conception interval for the herd was 200.78 ± 15.97 days.

The results indicate that treatment with GnRH and PGF2α during early postpartum period is useful for improving the reproductive efficiency and reducing the intercalving interval of the herd. Among the various oestrus synchronization protocols, single $PGF_2\alpha$ administration on confirmation of a functional CL by an expert was found to be the most economical in small herds. The treatment cost involved is meager when compared to the loss incurred due to increased number of days open and cost incurred for replacement stock. Hence it is recommended that Ovsynch and prostaglandin protocols can be effectively employed according to the ovarian status for improving the reproductive efficiency in post partum dairy cows.

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Abstract

SYNCHRONIZATION OF OVULATION AND TIMED ARTIFICIAL INSEMINATION TO IMPROVE FERTILITY IN POSTPARTUM DAIRY COWS

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ABSTRACT

The present research work was undertaken to evaluate different oestrus synchronization protocols and to recommend a better and more consistent timed artificial insemination (TAI) protocol for improving fertility in postpartum dairy cows. The study was performed in 30 crossbred cows at day 40 postpartum belonging to University Livestock Farm, Mannuthy during the period from July 2007 to May 2008. Efficacy of various synchronization protocols for inducing oestrus and ovulation and conception rate in the experimental and control groups were determined.

In Group I, 20µg of GnRH analogue (Buserelin) was administered intramuscularly on day 40 postpartum followed by 500µg of $PGF_2\alpha$ analogue (Cloprostenol) on day 7 intramuscularly and a second dose of 10µg GnRH was administered on day 9 followed by TAI at 24^{th} and 32^{nd} hours. In Group II, 20µg of GnRH analogue was administered intramuscularly on day 40 postpartum followed by 500µg of $PGF_2\alpha$ analogue intramuscularly on day 11 and a second dose 10µg GnRH analogue was administered on day 13 followed by TAI at 24^{th} and 32^{nd} hours. In Group III, induction of oestrus was done by administering $PGF_2\alpha$ analogue 500µg intramuscularly on day 40 postpartum. A second dose of $PGF_2\alpha$ analogue was administered on day 11, followed by TAI at 72^{nd} and 80^{th} hour. In Group IV, cows with a palpable functional CL on day 40 postpartum were administered 500µg $PGF_2\alpha$ analogue and were inseminated at observed oestrus. Cows inseminated during first natural post partum oestrus formed the control group (Group V). In the experimental and control groups serum progesterone was estimated on day 40 postpartum, on days of hormone administration and during oestrum. Pregnancy diagnosis was done by rectal palpation of genitalia at 60 days after AI in all groups and the data were subjected to statistical analysis.

Response to oestrus synchronization was 83.33, 33.33, 66.67 and 100 per cent in Group I to IV respectively. The time taken for induction of oestrum was 52.50 ± 0.99, 52.33 ± 0.71, 52.83 ± 1.40 and 53 ± 0.97 h respectively in Group I to IV but there was no significant difference between the groups. The duration of oestrus in Groups I to V were 37.33 ± 0.71, 35.67 ± 0.88, 40.50 ± 0.76, 38.83 ± 0.83 and 39.83 ± 0.48 h respectively.

The percentage of animals showing high, medium and low intensities of oestrum respectively were 0, 33.33 and 50 in Group I, 0, 33.33 and 0 in Group II, 33.33, 33.33 and 0 in Group III, 83.33, 16.66 and 0 in Group IV, 83.33,16.66 and 0 in Group V.

The mean serum progesterone level on day 0, 7, 9 and observed oestrum for those conceived in Group I were1.56 ± 0.69, 5.00 ± 0.94, 0.55 ± 0.09 and 0.36 ± 0.06 ng/ml. For those animals in Group I that did not conceive, the corresponding values for the same days were 0.36 ± 0.01, 0.76 ± 0.61, 0.40 ± 0.11 and 0.32 ± 0.08 ng/ml respectively. In Group II, the mean serum progesterone level on day 0, 11, 13 and observed oestrum for the conceived were 0.49 ± 0.23, 1.59 ± 0.59, 0.35 ± 0.13 and 0.88 ± 0.13 ng/ml respectively and those that did not conceive had mean serum progesterone level 0.99 ± 0.44, 0.35 ± 0.20, 0.31 ± 0.07 and 0.88 ± 0.21 ng/ml respectively. In Group III, the mean serum progesterone level on day 0, 11 and observed oestrum for the conceived were 0.83 ± 0.19, 3.05 ± 0.38 and 0.33 ± 0.10 ng/ml respectively and for the non conceived the corresponding values were 0.20 ± 0.05, 0.34 ± 0.11 and 1.39 ± 0.01 ng/ml respectively. The conceived animals in Group IV had mean serum progesterone level 2.77 ± 0.38 and 0.46 ± 0.12 ng/ml respectively on day 0 and observed oestrum while for those that did not conceive the corresponding values were 1 and 0.5ng/ml respectively. In the control group those that conceived had serum progesterone levels 1.30 ± 0.29 and 0.55 ± 0.08 ng/ml on day 40 and observed oestrum and the corresponding values for those that did not conceive were 1.6 ± 0.85, 0.685 ± 0.235 ng/ml respectively.

The conception rates after synchronization in Groups I to V were 66.66, 33.33, 66.66, 83.33 and 66.66 per cent respectively. The overall conception rate in Groups I to V were 83.33, 83.33, 66.66, 83.33 and 66.66 per cent respectively.

The mean calving to conception interval for the experimental groups was 53.84 ± 2.31 days whereas the corresponding values for the control and herd were 95 ± 6.19 and 200.78 ± 15.97 days respectively.

The present study revealed that treatment with GnRH and $PGF_2\alpha$ during early post partum period was useful for reducing the intercalving interval in a herd. Similarly single $PGF_2\alpha$ administration on confirmation of a functional CL by clinical examination was useful and more economical for individual animals and small herds. Hence it is recommended that Ovsynch and $PGF_2\alpha$ protocol could be suitably employed for reducing the intercalving period in post partum dairy cows.