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REPRODUCTIVE PERFORMANCE OF DAIRY COWS FOLLOWING DIFFERENT ESTROUS SYNCHRONIZATION PROTOCOLS

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INFORMAN

Que KAILASAM MURUGAVEL ha realizado bajo nuestra dirección el estudio titulado: "*Reproductive performance of dairy cows following different estrous synchronization protocols*", con la finalidad de optar al grado de Doctor en Veterinaria por la Universidad Autónoma de Barcelona.

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REVIEW OF LITERATURE

LUTEAL ACTIVITY AT THE ONSET OF A TIMED INSEMINATION PROTOCOL AFFECTS REPRODUCTIVE OUTCOME IN EARLY POSTPARTUM DAIRY COWS

ABBREVIATIONS

AI	Artificial insemination
CL	Corpus luteum
EB / OBD	Estradiol benzoate
eCG / PMSG	Equine chorionic gonadotropin / Pregnant mare serum gonadotropin
FSH	Follicle stimulating hormone
g	Gram
GnRH	Gonadotropin releasing hormone
h	Hour
hCG	Human chorionic gonadotropin
im	Intramuscular
inj.	Injection
IU	International units
IVSM	Intravulvosubmucosal
Kg	Kilogram
LH	Luteinizing hormone
mcg / µg	Microgram
mg	Milligram
MGA	Melengestrol acetate
min	Minute
mL	Milliliter
mm	Millimeter
MPA	Medroxyprogesterone acetate
m-RNA	Messenger - ribonucleic acid
ng	Nanogram
P4	Progesterone
PGF2α	Prostaglandin F_2 alpha
RIA	Radio immuno assay
TAI	Timed artificial insemination
VWP	Voluntary waiting period
wk	Week

SUMMARY

Reproductive efficiency is often a limiting factor in dairy herd productivity and profitability. A 12 to 13 month calving interval is habitually recommended to be optimal for a high annual milk yield and economic worth to dairy producers. The reproductive performance of postpartum dairy cows is frequently limited by factors like failure to ovulate or display estrus, along with poor estrus detection. In addition to this, the calving interval and interval to first service are highly correlated and optimal calving intervals may not be attainable without decreasing the intervals to first service. Thus, estrus detection and interval to first service, which includes an elative waiting period before first AI, are important factors that determine the reproductive efficacy of a dairy farm. Moreover, prolonged postpartum anestrus, a common cause for prolonged intercalving period in dairy cows, is primarily due to the combination of a delayed interval to first estrus, silent estrus after parturition and a poor detection of estrus. The situation is further aggravated by a high incidence of ovarian disorders in high milk yielding early postpartum dairy cows.

In order to improve the estrus detection rate, many treatment protocols have been proposed to speed up the return to normal ovarian cyclicity after parturition, and to synchronize ovulation for timed insemination in dairy cows. But detailed investigations on estrous synchronization programs for early postpartum dairy cows especially with ovarian disorders are a few in number.

With the general objective to improve reproductive performance in early postpartum dairy cows, the present research work has been developed to evolve and recommend a better and a more consistent timed insemination estrous synchronization program for postpartum dairy cows, including cows with ovarian disorders, without compromising on the pregnancy rates.

In the first experiment, the effects of presynchronization on subsequent ovarian activity in clinically normal lactating dairy cow intervals was studied with a double dose of prostaglandins at 14 days during the preservice period. Depending on the chronological order of parturition, the cows were alternately assigned to a control (n=102) or treatment (n=101) group. Animals in the treatment group were administered 2 cloprostenol treatments 14 d apart, beginning on Day 22 postpartum. The follicular persistence rates were similar in the presynchronized (14.9 %) and control (13.7 %) groups. Cows in the presynchronized group showed a lower metritis-pyometra rate (0 % < 3.9 %; P = 0.045); a lower ovarian cyst rate (3 % < 10.8 %; P = 0.03); a higher luteal activity rate (progesterone > 1 ng/mL) on Day 50 postpartum (76.2 % > 52.9 %; P = 0.0005); a higher estrus detection rate (73.3 % > 47.1 %; P < 0.0001); a higher ovulation rate (72 % > 44 %; P < 0.0001) and a higher pregnancy rate (29.7 % > 15.7 %; P = 0.02) than controls.

The second study was designed to compare two timed insemination protocols, the progesterone, GnRH and PGF2α combination protocol and the Ovsynch protocol, in presynchronized, early postpartum dairy cows. Cows in the control group (Ovsynch, n=30) were treated with Ovsynch protocol. Cows in group PRID (n=45) were fitted with a progesterone releasing intravaginal device (PRID) for 9 d, and were given GnRH at the time of PRID insertion and PGF2α on Day 7. In group PRID/GnRH (n=31), cows received the same treatment as the PRID group, but were given an additional GnRH injection 36 h after PRID removal. The cows were inseminated 16 to 20 h after the administration of the second GnRH dose in the Ovsynch group, and 56 h after PRID removal in the PRID and PRID/GnRH groups. In cows with a high progesterone concentration at treatment onset, Ovsynch treatment resulted in a significantly improved pregnancy rate over values obtained following PRID or PRID/GnRH treatment. In cows with low progesterone concentration, PRID or PRID/GnRH treatment led to a markedly increased ovulation and pregnancy rate with respect to Ovsynch treatment.

The third study was designed to compare the reproductive performance of presynchronized postpartum dairy cows subjected to either the Ovsynch protocol without screening for ovarian status, or to a specific estrous synchronization protocol applied according to their ovarian status, as determined by transrectal ultrasound. The study was conducted on 428 lactating dairy cows presynchronized with 2 cloprostenol im treatments given 14 d apart, starting from Day 14 to 20 postpartum. The cows were then assigned to one of the two treatment groups. Cows in the Ovsynch group (n=205) received GnRH im, on

Day 0; cloprostenol im, on Day 7; GnRH im, 36 h later; AI 16 to 20 h after the second GnRH. Cows in the specific synchronization (Ssynch) group (n=223) were weekly subjected to transrectal ultrasound exams for 4 weeks, or until AI or till the start of treatment, and then divided into four subgroups according to their ovarian status: 1) CL subgroup (n=130), cows with a corpus luteum. These cows received 500 µg im cloprostenol and 250 IU hCG plus 1 mg EB im 12 h later, and were inseminated 48 h after cloprostenol treatment; 2) NE subgroup (n=58), cows inseminated at natural estrus; 3) PF subgroup (n=26), cows considered to suffer follicular persistence. This subgroup was treated with 1.55 g intravaginal progesterone (PRID) for 9 d; 100 µg GnRH im on Day 0; 500 µg cloprostenol im on Day 7; AI 56 h after PRID removal; and 4) OC subgroup (n=9), cows with ovarian cysts. These cows were given 100 µg GnRH plus 500 µg cloprostenol im on Day 0; 500 µg cloprostenol im on Day 14 followed by 100 µg GnRH im 36 h later; AI 24 h after the second GnRH dose. There were no significant effects of treatment regime on ovulation rate, nor were there any effects of lactation number, milk production and body condition on pregnancy rates. Insemination season was a significant risk factor for pregnancy to first and to second AI. The results of this study show that cows undergoing specific synchronization were 2.1 times more likely to become pregnant to first and second AI, compared to those synchronized using the Ovsynch protocol. Weekly veterinary supervision of ovarian status before applying a program of estrous synchronization and timed AI has found to improve the reproductive performance in postpartum dairy cows.

In conclusion, presynchronization during the preservice period improves ovarian activity from Days 50 to 71 postpartum along with pregnancy rates in dairy cows. Luteal activity, at the time of onset of timed insemination estrous synchronization protocol influences subsequent reproductive performances in lactating dairy cows. Adopting a specific estrous synchronization protocol applied according to the ovarian status rather than applying a single protocol regardless of ovarian status of the cows can improve reproductive performance of the early postpartum dairy cows.

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I. INTRODUCTION

The success of any scientific research depends on the effects it has on humanity. Thus, the human edge is definitely not to be ignored especially as the rapidly expanding world population is bringing about the depletion of resources at an alarming rate. This critical position has made it mandatory for research scholars to develop faster and more efficient means of obtaining the basic necessities of man without compromising on quality.

In this intricate web, animal production has an important role to play as foods of animal origin represent about one-sixth of human food energy and one-third of the human food protein on a global basis. In this ratio, milk and its products by playing a formidable role in human nutrition, have made theriogenologists to play a pivotal role in developing technologies to improve the reproductive efficiency in dairy cattle in turn to increase the efficiency and profitability of milk production.

Of the methods widely used in animal husbandry practice for increasing reproductive potential, artificial insemination is perhaps the most important technique that has proved to be exceedingly effective for breeding dairy cattle especially, as the economic advantages of artificial insemination when compared to natural mating are very great (Polge, 1972). However, problem associated with accurate estrus detection in dairy cows, especially during early postpartum period, diminish the potential use of AI in dairy operation (Senger, 1994).

Several studies have documented the link between poor estrus detection and reproductive inefficiency (Barr, 1975; Britt, 1975). Reproductive efficiency is often a limiting factor in dairy herd productivity and profitability (Louca and Legates, 1968; Olds et al., 1979; Oltenacu et al., 1981; Hamudikuwanda et al., 1987). For maximum reproductive efficiency to be achieved in a herd of cattle, each cow must reproduce as frequently as possible. A 12 to 13 month calving interval is often recommended optimal for high annual milk yield and economic value to dairy producers (Holmann et al., 1984). Calving intervals longer than optimal, result in cows spending a greater proportion of their productive herd-life in the latter and less profitable stages of their lactation curve (Call, 1978).

In addition, calving interval and interval to first service are highly correlated (Britt, 1975; Harrison et al., 1974; Slama et al., 1976), and optimal calving intervals may be unattainable without decreasing intervals to first service (Call, 1978). Thus, estrus detection and interval to first service, which includes an elative waiting period before first AI, are important factors that determine the reproductive efficiency of a dairy farm (Lucy et al., 1986; Schneider et al., 1981).

Moreover, prolonged postpartum anestrus, a common cause for prolonged intercalving period in dairy cows, is primarily due to the combination of a delayed interval to first estrus, silent estrus after parturition and poor detection of estrus. After parturition, the first dominant follicle ovulates in many of the cows (70 to 80 %) (Savio et al., 1990) during 3 to 5 weeks postpartum (Zemjanis, 1961), even if the resumption of follicular growth occurs soon after calving (within 7 to 10 d of calving) (Savio et al., 1990). However, in a majority of normal cows (94%), this first ovulation occurs without behavioral estrus (Savio et al., 1990).

Since high yielding early postpartum dairy cows often suffer from one or another ovarian disorder (Opsomer et al., 2000; López-Gatius et al., 2002; Wiltbank et al., 2002), the situation is further aggravated during early postpartum period. In fact, regular cyclicity before 50 d postpartum is observed in only 51% of high yielding dairy cows; the risk factors calving season, problem calvings, clinical disease, ketosis or severe negative energy balance during the postpartum period are related to delayed cyclicity before service (Opsomer et al., 2000).

In order to improve the estrus detection rate, estrus synchronization programs using prostaglandin F2alpha (PGF2α) or progestogens that focus on controlling the lifespan of the corpus luteum have been implemented (Lucy et al., 1986; Chenault, 1992). Pregnancy rates were reported to be similar when dairy cows were bred at a detected estrus after synchronization of estrus with PGF2α or estrus after spontaneous estrus (Stevenson et al., 1987; Stevenson and Pursley, 1994). However, estrus was not synchronized precisely with PGF2α as this treatment does not synchronize growth of follicles but only regulates the lifespan of the corpus luteum. Thus, detection of estrus is needed over a period of 7 d after administration of PGF2α (Larson and Ball, 1992; Lucy et al., 1986). Consequently, when cows received fixed timed insemination following PGF2α treatment, pregnancy rates were considerably lower than those of cows receiving AI at a detected estrus (Stevenson et al., 1987; Stevenson and Pursley, 1994).

Recently a timed artificial insemination protocol (Ovsynch) based on the use of GnRH and prostaglandins to synchronize ovulation was developed for use in dairy cows (Pursley et al., 1995). This protocol synchronizes both follicular wave development and regression of the corpus luteum (Pursley et al., 1995). This program, extensively used at farm level (Nebel and Jobst, 1998), includes a GnRH treatment given at a random stage in the estrous cycle followed 7 d later by an injection of PGF2α. Thirty to thirty six hours later, a second dose of GnRH is administered and cows are inseminated 16 to 20 h after this last injection without detection of estrus. Further, there have been several recent reports of protocols in which prostaglandins are combined with other hormones for therapeutic estrus synchronization in early postpartum dairy cows with an ovarian disorder (Bartolome et al., 2000; López-Gatius et al., 2001; Pursley et al., 2001; López-Gatius and López-Béjar, 2002).

This scenario clearly shows the need for an in-depth study on estrus synchronization program for fixed timed insemination in early postpartum dairy cows, which includes cows with ovarian disorders with reference to Ovsynch protocol, so as to evolve and recommend a better and more consistent estrus synchronization program without compromising on pregnancy rates in postpartum dairy cows.

II. REVIEW OF LITERATURE

1. Introduction to synchronization of estrus in postpartum dairy cows.

1.1. Historical background of estrous synchronization in cattle.

The history of estrous cycle synchronization and the use of artificial insemination in cattle is a testament to how discoveries in basic science can be applied to advance the techniques used for livestock breeding and management (Beal, 2002).

The first successful synchronization of estrus in cattle was reported in 1948 (Christian and Casida, 1948). Since then more concentration was focused towards research on estrous synchronization and development of estrous synchronization products (Table 1). Synchronizing estrous cycles of domestic cattle depends on control of the functional life span of the corpus luteum (Hansel and Convey, 1983). There are two ways to facilitate control of the corpus luteum that result subsequently in estrus and ovulation. The first method involves long term administration of a progestin with subsequent regression of the corpus luteum during the time the progestin is administered (Britt, 1987). Estrus and ovulation occur within 2 to 8 days after progestin withdrawal. The second method involves the administration of a luteolytic agent that shortened the normal life span of the corpus luteum. This is accompanied generally with estrus and ovulation within 48 to 120 h after injection.

Table. 1. Chronology of significant developments in the applied endocrinology in animal reproduction with reference to estrus synchronization.

Year	Landmarks
1903	Ludwig Fraenkel observed that corpus luteum is essential for maintenance of pregnancy in rabbit.
1923	Allen and Doisy isolated and synthesized estrogen
1927	Hammond reported that removal of the corpus luteum from the cow's ovary is followed within a few days by estrus and ovulation (Hammond, 1927).

1927 Ascheim and Zondek reported the detection of hCG by bioassay in pregnant women.

1929 Corner and Allen isolated and synthesized progesterone.

1930 Cole and Hart, first demonstrated the presence of gonadotropin substance in the serum of pregnant mare.

1930 Kurzrok and Lieb observed contraction of uterus that addition of human seminal fluid

1935 Willard Allen Coined the word “progesterone” for the substance secreted from corpus luteum that maintains pregnancy.

1937 Makepeace and others demonstrated that administration of exogenous progestins to control estrus and ovulation in rabbits.

1937 Ulf Von Euler named the extract of the seminal vesicle of sheep, which contract the smooth muscles as “prostaglandin”.

1940s Geoffrey Harris and coworkers proposed that hypothalamus regulates the secretion of the anterior pituitary gland by liberating substances.

1948 Christian and Casida record the successful synchronization of estrous cycles in cattle by using daily injections of progesterone.

1960 Hansel and Malven, first used orally active progestins to synchronize estrus and ovulation in cattle.

1964 Kaltenbach et al. reported the luteolytic property of estrogen.

1966 Mauléon and Rey administered progestagens intravaginally by means of impregnated sponge pessaries.

1966 Dziuk and coworkers used silastic implants containing Melengestrol acetate for synchronization of estrus in cows.

1966 Babcock first suggested that prostaglandin might be a luteolytic agent.

1969 Phariss and Wyngarden reported that prostaglandin is luteolytic in rats.

1971	Barrett and co-workers reported that PGF2α of uterine origin is luteolytic factor in ruminant (Ewe).
1971	Schally et al reported that GnRH from hypothalamus regulates the release of LH and FSH and they also isolated GnRH from porcine hypothalami.
1971	Baba and others established the molecular structure of the GnRH.
1972	Several workers reported the luteolytic action of prostaglandin F2α in cattle.
1974	Report of cloprostenol, a synthetic prostaglandin by Binder and coworkers.
1974	Cooper and Furr reported the luteolytic effect of cloprostenol, a prostaglandin analogue in cattle.
1973	Schams and others. demonstrated that administration of GnRH induces ovulation.
1976	Niswender and others proposed the mechanism of luteolytic action of prostaglandin.

1.1.1. Hormones used in estrous synchronization.

Synchronization of estrous cycle is cows usually based on prostaglandin or its analogues (fatty acids having hormone-like properties) and progestagens (steroid hormones). To improve the efficacy of synchronization protocols based on progesterone and/or PGF2α, follicular growth and corpus luteum regression are synchronized by administration of estrogens, GnRH and its agonists (steroid and polypeptide hormones, respectively). Moreover, some of the estrous synchronization protocols include preparations of placental gonadotropins, especially equine chorionic gonadotropin (glycoprotein), which is rich in FSH activity, and human chorionic gonadotropin (glycoprotein), which is rich in LH activity.

1.1.1.1. Progestagens

The history of progesterone dates from as far as 1903 when Ludwig Fraenkel, a young gynaecologist from Breslau found removal of corpus lutea from rabbits a few days after mating prevented pregnancy. This was the first evidence that the ovaries contributed anything to pregnancy other than eggs. The name "progesterone" was coined by Willard Allen of Rochester, New York (Heap and Flint, 1979). Isolation and synthesis of progesterone was first reported in 1929 (Corner and Allen, 1929). Administration of exogenous progestins to control estrus and ovulation has evolved since 1937 when Makepeace et al. (1937) demonstrated that progesterone injections inhibited ovulation in rabbits. Christian and Casida (1948), using daily injections of progesterone, were the first to record the successful synchronization of estrous cycles in cattle. Hansel and Malven (1960) first reported the use of orally-active progestins to synchronize estrus and ovulation in cattle in 1960. Subsequently, various forms and methods of administration of progestins were tested and generally shown to be effective at synchronizing estrus (Trimberger and Hansel, 1955; Hansel and Fortune, 1978). Progestagens had been administered intravaginally by means of impregnated sponge pessaries, which, in theory, permit a more precise treatment of individual animals (Mauléon and Rey. 1966; Carrick and Shelton, 1967). Dziuk et al. (1966) and Dziuk and Cook (1966) used silastic implants containing MGA (Melengestrol acetate) for synchronization of estrus by inserting the implant in the neck region of cows. Curl et al. (1968) reported good estrous synchronization rates using subcutaneous implants containing norethandrolone.

Later, short-tern progestagen treatments in combination with estradiol were administered by means of norgestomet (17 alpha-acetoxy-11-beta-methyl 19-nor-preg-4-ene, 20-dione) ear implant for 9 days (Wishart and Young, 1974; Wiltbank and Gonzalez-Padilla, 1975)) or by norgestomet in silastic coated coils, the progesterone releasing intra-vaginal device (PRID) (Roche, 1974a) or by silicone rubber impregnated with progesterone, controlled intravaginal drug release (CIDR) (Macmillan and Peterson, 1993).

1.1.1.2. Estrogens.

Following the isolation and synthesis of estrogen (Allen and Doisy, 1923), studies that demonstrated estradiol as a luteolytic agent when administered early in the bovine estrous cycle (Kaltenbach et al, 1964; Wiltbank, 1966) was established. In the cows, administration of estrogens can induce a preovulatory like LH surge, ovulation (Lammoglia et al., 1998), and can exert luteolytic activity during the luteal phase (Salfen et al., 1999). Estrogens have been shown to induce follicle atresia (Hutz et al., 1988), and the effects of estrogens on gonadotropins and preovulatory follicles have been reported in several studies (Engelhart et al., 1989; Rajamahendran and Walton, 1990). In recognition of the luteolytic properties of estradiol and its incorporation into short term (9-12 day) treatments with progestagens has been reported to produce normal fertility during the synchronized estrus (Wiltbank and Kassan, 1968). Subsequently, estradiol was also used during proestrum following prostaglandin F2α treatment to improve conception rate in cattle (Welch et al., 1975).

1.1.1.3. Human Chorionic Gonadotrophin (hCG).

In 1927, scientists from Germany first detected human chorionic gonadotrophin (hCG) in pregnancy women by bioassay (Ascheim and Zondek, 1927). Human chorionic gonadotrophin has been detected in the urine of pregnant women as early as 8 d after conception by sensitive radio immunoassays (Jeffe, 1978). Human chorionic gonadotrophin, a glycoprotein (Bahl, 1978), has both LH- and FSH-like actions, but predominately LH-like biologic actions (Reeves, 1987). In pregnant woman, it is associated with prolongation of the lifespan of the corpus luteum and therefore with the maintenance of pregnancy (Hunter, 1980). Administration of hCG in regularly cycling dairy cows induce ovulation of the dominant follicle within 48 h of treatment (Rajamahendran and Sianangama, 1992). Several scientists have also shown the effectiveness of hCG in inducing ovulation and forming a functional corpus luteum (Price and Webb, 1989; Fricke et al., 1993; Sianangama and Rajamahendran, 1996). Because of the above observations, hCG was used usually during preovulatory period in estrous synchronization programs to achieve a good synchrony and high pregnancy rates (López-Gatius, 1989; López-Gatius and Vega-Prieto, 1990)

1.1.1.4. Equine Chorionic Gonadotropin (eCG)

Cole and Hart (1930) first demonstrated the presence of a gonadotropin substance in the serum of pregnant mare. Pregnant mare serum gonadotropin (PMSG) is a glycoprotein hormone secreted by the endometrial cups of equines (Cole and Goss, 1943). However, Allen and Moor (1972) demonstrated that the endometrial cups that produce PMSG were of placental origin and a proper designation for this hormone is equine gonadotrophin (eCG). Following its initial appearance in the blood between Days 37 and 40 of gestation, eCG concentrations rise rapidly to a well-defined peak between Days 55 and 75 and thereafter decline steadily to become undetectable again between Days 120 and 150 (Allen, 1969). It was found to possess both FSH and LH biological activities within the one molecule, but the former predominates (Gospodarowicz, 1972). The FSH like activity of eCG was utilized for follicular stimulation in the progestogen based estrous synchronization program in cattle (Kastelic et al., 1999; Humblot et al., 1996). Treatment with eCG at the time of removal of progesterone treatment is often recommended, especially if a high proportion of the cattle are in anestrus (Munro and Moore, 1985; Tregaskes et al., 1994).

1.1.1.5. Prostaglandin F2alpha (PGF2α)

The history of prostaglandin began when two New York American gynaecologists, Kurzrok and Lieb noted the contraction of uterus to the addition of human seminal fluid in 1930 (Baird, 1972; Challis 1979). Nobel Laureate Ulf Von Euler of Sweden, in 1937 found extracts of the seminal vesicles of sheep stimulated strong contractions in smooth muscles. He named this lipid soluble acid fraction, which contained this biological activity "prostaglandin" because he thought it came from the prostate gland (Challis, 1979; Lauderdale, 2002). Prostaglandin F2α is the most often discussed prostaglandin relative to domestic animal research and practical utility. In 1966, Babcock apparently made the first suggestion that prostaglandins might be a luteolytic agent (Hansel and Blair, 1996). This suggestion was followed by the discovery by Phariss and Wyngarden (1969) that prostaglandin F2α is luteolytic in rats. It was not until 1971 that work by Goding and co-workers established that PGF2α of uterine origin is a luteolytic factor in the ewe (Barrett et al., 1971; Goding et al., 1972). Niswender et al. (1976) proposed the most probable hypothesis of how PGF2α induces corpora lutea regression. They have evidence that venoconstrictive effects of PGF2α may induce hypoxia, which in turn leads to luteolysis. By 1972, several groups (Rowson et al., 1972; Hansel and Schechter, 1972; Louis et al., 1972; Liehr et al., 1972) reported that prostaglandin F2α is luteolytic in the cow when given between Days 5 to 16 of the estrous cycle. Following the report of cloprostenol, a synthetic prostaglandin structurally related to natural PGF2α (Binder et al., 1974), Cooper and Furr (1974) recorded the luteolytic activity of the drug in cattle at a single intramuscular dose of 500μg. The mechanism by which PGF2α gets from the endometrium of the uterus to the ovary is unique in that PGF2α passes directly through the walls of the utero-ovarian vein into the ovarian artery and directly to the corpus luteum (McCracken, 1980).

1.1.1.6. Gonadotropin Releasing Hormone (GnRH).

Geoffrey Harris and his co-workers pioneered the earliest known work done on GnRH in the 1940s and 1950s. From their observations, they postulated that the hypothalamus regulated the secretions of the anterior pituitary gland by liberating substances, which were carried to the pituitary via the hypophysial portal blood vessels (Fraser, 1979).

In 1971, it was reported that a polypeptide namely, gonadotropin-releasing hormone from hypothalamus, was found to regulate the secretion of luteinizing and follicle stimulating hormones (Schally et al., 1971a). In the same year, LH and FSH-releasing hormone was isolated from the porcine hypothalami (Schally et al., 1971b) and its molecular structure was established (Baba et al., 1971). The Induction of ovulation of ovarian follicles was demonstrated in milked (Britt et al., 1974) and suckled cows (Schams et al., 1973) following an injection of Gonadotropin-releasing hormone. GnRH induced effects is indirect (Chenault et al., 1990) through their induced release of luteinizing hormone (LH) (Britt et al., 1974) and follicle stimulating hormone (FSH) (Foster et al., 1980) from anterior pituitary gland. Later, GnRH analogues and agonist were developed, which were more potent than native GnRH (Thatcher et al., 1993). Synchronization of follicular waves and selection of new large follicle following GnRH at any stage of the estrous cycle was used as a tool to further develop estrous synchronization programs for fixed timed AI (Twagiramungu et al., 1995a).

2. Physiological basis for estrus synchronization.

2.1. Follicular dynamics in dairy cattle.

The follicular development in the ovary of the dairy cattle is a wave-like dynamic sequence of organized events under hormonal control (Pierson and Ginther, 1988; Savio et al., 1988; Knopf et al., 1989). A follicular wave has been defined as a synchronous development of several follicles 4 to 5 mm in diameter, followed by selection and growth of the dominant follicle and subsequent regression of the remaining subordinate follicles (Ginther et al., 1989a, b). The estrous cycle includes of two or three follicular waves in most of the dairy cows. In case of a two wave cycle, the emergence of follicular waves usually take place on the day of ovulation (Day 0) and on Day 10 of the estrous cycle. Whereas, in case of a three follicular wave cycle, the emergence of follicular waves develop on Day 0, 9, and 16 of the estrous cycle (Ginther et al., 1989a, b). A great variation in the different proportions of cows exhibiting two or three follicular waves during the estrous cycle, and in the day of follicular wave emergence, particularly the day of emergence of the second follicular wave has been reported (Bo et al., 1995a). This variation in the follicular wave dynamics has been attributed to the influence of various factors like genetic and environmental factors (Bo et al., 1995a).

2.2. Follicular dynamics in postpartum dairy cows.

In most of the lactating dairy cattle with uncomplicated parturitions, the first post-partum estrus accompanied by ovulation occurs as short as 15 d postpartum, although longer than 100 d have been recorded (Murphy et al., 1990; Mawhinney et al, 1996). The cows are in anovulatory period for a variable period of time from the regression of the corpus luteum (CL) of pregnancy to first ovulation. The length of this period depends on numerous factors such as level of nutrition, body condition, dystocia, breed, age, season, uterine pathology and chronic debilitating disease (Zemjanis, 1961; Lamming et al., 1981; Tuker, 1982; Short et al., 1990; Roche and Boland, 1991; Stagg et al., 1995).

The follicles with 6 to 8 mm in diameter begin to grow within Day 7 to 10 of calving. A single dominant follicle emerges from these recruited follicles and ovulates between Day 10 to 30 postpartum. One to three follicular waves can be observed before first ovulation in postpartum dairy cows (Savio et al., 1990). The first dominant follicle ovulates in many of the cows (70 to 80 %) but in majority of cows (94 %), this ovulation was often not accompanied by overt behavioral estrus (King et al., 1976; Savio et al., 1990). Even though, the resumption of follicular growth starts soon after calving, the first ovulation takes place 3 to 5 wk postpartum (Zemjanis, 1961). Failure of ovulation of the dominant follicle is the major cause for the anoestrus condition in postpartum cows (Opsomer et al., 2000; Wiltbank et al., 2002) and may be due to insufficient pituitary stores of LH (Nett, 1987) or variable period of refractoriness to the stimulatory effects of estradiol-17 β on LH secretion in early port-partum period (Schallenberger and Prokopp., 1985). Further, Low progesterone concentrations related to subluteal activity, have been associated with intermediate LH pulse frequencies, maintaining estradiol production by the dominant follicle which does not ovulate and become persistent (Savio et al. 1993; Stock and Fortune, 1993).

2.3. Principles behind estrus synchronization in dairy cattle.

Most of the early studies in the sixties, on controlling the estrus in cattle, used natural steroids, progesterone, and it become clear that although estrus and ovulation could be controlled with some degree of accuracy, conception rate at synchronized estrus was often unacceptably low. Later satisfactory methods become available in the 1970's with the advent of prostaglandin F2 alpha (PGF2α) (and its analogues) and short – term progesterone / progestogen treatments (Jochle, 1993; Gordon, 1996).

The two main basic approaches to controlling estrus in the cows with acceptable fertility levels were either to prolong the luteal phase of the estrous cycle artificially using progesterone or progestogen or to shorten the estrous cycle by means of the luteolytic action of prostaglandins (Diskin and Sreenan, 1994).

This review will focus on the recent developments in methods to control estrous cycle in dairy cattle with special reference to lactating postpartum dairy cows.

3. Prostaglandin and its analogues treatment in postpartum dairy cattle.

3.1. Prostaglandin in synchronization of estrus in dairy cows.

In the early 1970s several workers pioneered the luteolytic effect of prostaglandin F2α (PGF2α) in cattle (Lauderdale, 1972; Liehr et al., 1972; Louis et al., 1972; Rowson et al., 1972). Subsequent research efforts then attempted to improve the reproductive efficiency of dairy cattle by inducing estrus with PGF2α (Lauderdale et al., 1974; Louis et al., 1974; Leaver et al., 1976; Roche, 1976a; Macmillan, 1978; Seguin et al., 1978; Plunkett et al., 1984). Several studies demonstrated the capacity of PGF2α and its synthetic analogues, alfaprostol (Jochle et al., 1982; Schams and Karg, 1982; Randel et al., 1988; Tolleson and Randel, 1988; Randel et al., 1996), cloprostenol (Cooper, 1974; Cooper and Rowson, 1975), fenprostalene (Martinez and Thibier, 1984; Stotts et al., 1987), luprostiol (Godfrey et al., 1989; Plata et al., 1989; Plata et al., 1990) and tiaprost (Schams and Karg, 1982) to trigger the regression of a mature CL in the ovary, thus provoking and synchronizing estrus (Lauderdale et al., 1974; Macmillan and Day, 1982; Seguin et al., 1983; Stevenson et al., 1989; Stevenson and Pursley, 1994). When PGF2α was administered to cows with a functionally mature CL, 85 to 95% reached estrus within 7 d of treatment (Macmillan and

Henderson, 1983; Armstrong et al., 1989; Folman et al., 1990; Rosenberg et al., 1990); 70 to 90% showing signs of estrus 3 to 5 d after treatment (Ferguson and Galligan, 1993).

For PGF2α treatment to achieve its luteolytic effects, the cows must be in the diestrus stage of the estrous cycle (Day 7 to 17). Prostaglandin treatment in the early stage of estrous cycle (first 5 d) was found to be ineffective in causing a luteolytic response in cattle (Cooper and Rowson, 1975; Lauderdale, 1975). Consequently, a double protocol in which PGF2α was given at a 7, 11 or 14 d interval was developed so that cows at a stage in the estrous cycle other than diestrus would have a functional CL when they received the second PGF2α dose (Rosenberg et al., 1990; Baishya et al., 1980; Kristula et al., 1992). Kristula et al. (1992) reported that weekly doses of PGF2α allowed AI to be performed earlier, because cows not in the diestrus stage when subjected to the first PGF2α injection were found to have a functional CL when the second PGF2α injection was given 7 d later. However, several authors report the improved reproductive efficiency of cows detected to be in estrus after the second PGF2α dose using the double regime in which PGF2α doses are given 11 or 14 d apart (Folman et al., 1990; Ferguson and Galligan, 1993; Stevenson et al., 2000). Further, an enhanced estrus response and normal fertility were reported when PGF2α was given at the late, rather than early to middle stage of the luteal phase (Tanabe and Hann, 1984; Watts and Fuquay, 1985; Xu et al., 1997). Thus, the 14 d interval double prostaglandin regimen seems to show an improved response over the 11 d protocol, since two treatments given 14 d apart ensures that most animals are in the late luteal stage (cycle Day 11 to 14) when they receive the second PGF2α dose (Folman et al., 1990; Rosenberg et al., 1990; Young, 1989).

Recently, the successful use of a new estrus synchronization protocol for lactating dairy cows has been described, in which three PGF2α doses are given (Nebel and Jobst, 1998). In this protocol, known as the Targeted Breeding Program, all the animals that were not detected to be at estrus following the first PGF2α treatment were treated with a further two doses of PGF2α at 14 d intervals until artificial insemination at detected estrus or until timed artificial insemination was performed 72 to 80 h after the third PGF2α dose.

3.2. Fertility following prostaglandin induced estrus in dairy cattle.

Several researchers have noted normal or above normal fertility following synchronization of estrus with PGF2α in cows (Macmillan and Day, 1982; McIntosh et al., 1984; Lucy et al., 1986; Wenzel, 1991). Young and Henderson (1981) found no significant difference in conception rates after a double 11 d interval treatment regime using a prostaglandin analogue among cows inseminated only once at the fixed time of 75 to 80 h (46%), cows inseminated twice at 72 and at 96 h (47%) and control untreated cows (50%). Neither were differences found in cows timed AI following double 14 d-PGF2α treatment compared to natural estrus (Macmillan et al., 1977; Roche and Prendiville, 1979). However, reduced conception rates due to variations in the time of ovulation have been noted after timed AI, either following single (Fetrow and Blanchard, 1987; Archbald et al., 1992) or double (Waters and Ball, 1978; Stevenson et al., 1987) prostaglandin administration, compared to AI at detected estrus. Reproductive performance in dairy cattle was also improved following double 14 d-PGF2α treatment without assessing ovarian status when compared to a single dose based on detecting a CL by rectal palpation or by milk progesterone enzyme immunoassay (Heuwieser et al., 1997). Tenhagen et al. (2000) observed that timed insemination following double 14 d-prostaglandin treatment reduced the number of days open in lactating dairy cows when compared to AI performed at observed estrus.

3.3. Prostaglandin treatment during early postpartum period.

There is evidence that PGF2α is capable of improving the reproductive performance of dairy cows when given before the end of the voluntary waiting period (Pankowski et al., 1995). Administering PGF2α during the early postpartum period led to increased first service conception rates related to the associated benefits of enhancing uterine activity (Young et al., 1984), thereby decreasing the interval between calving and conception (Etherington et al., 1984; Benmrad and Stevenson, 1986). However, others suggest that the diminished intercalving period may be an effect of luteolysis and an increased number of estrous cycles (Thatcher and Wilcox, 1973; Young, 1983). In a meta-analysis, Burton and

Lean (1995) explored the effects of prostaglandin given in the early postpartum on the subsequent reproductive performance of dairy cattle. Their pooled data corresponded to 21 independent trails performed on 2646 cows described in 10 papers. Meta-analysis of the effect of prostaglandin treatment during the early postpartum period revealed no increase in pregnancy rate to first artificial insemination in cows with a normal or abnormal puerperium, while the period from calving to first AI was significantly reduced, thus reducing the number of days open in the dairy farm. These results were however not considered conclusive by the authors.

3.4. Factors influencing the effects of prostaglandin treatment.

3.4.1. Stage of estrous cycle at the time of prostaglandin treatment.

Since, induction of estrus was brought about by the luteolytic effect of PGF2α on the mature CL, the success of PGF2α primarily depends on the presence of a mature functional CL in the ovary. (Kristula et al., 1992). Therefore, the stage of estrous cycle at the time of administration of the drug influence the ability of prostaglandin to induce luteolysis in cows (Cooper, 1974; Leaver et al., 1975; Johnson, 1978; Jackson et al., 1979; Hansen et al., 1987).

The stage of follicular wave development at the time of PGF2α treatment appears to be the factor determining the time of estrus onset (Kastelic et al., 1990; Twagiramungu et al., 1992; Ferguson and Galligan, 1993; Adams, 1994; Twagiramungu et al., 1995a). Thus, the time elapsed between PGF2α treatment and the onset of estrus depends on the stage of the estrous cycle at the time of PGF2α treatment (Roche, 1974b; Johnson, 1978; Jackson et al., 1979; King et al., 1982; Macmillan and Henderson, 1983; Stevenson et al., 1984; Tanabe and Hann, 1984; Voh et al., 1987a,b). The mean interval to estrus was 48 to 72 h when PGF2α was administered on estrous cycle Day 5 or Day 8 in dairy cows (Tanabe and Hann, 1984; Watts and Fuquay, 1985). Prostaglandin administration in mid-cycle (Day 8 to 11) or later in the luteal phase (Day 12 to 15) resulted in a mean time to estrus of 70 and 62 h, respectively (King et al., 1982; Stevenson et al., 1984).

Similar response in heifers was observed by Stevenson et al. (1984), who reported that the heifers comes to estrus 11 h earlier when prostaglandin was administered during Day 5 to 8 of estrous cycle when compared to, treatment given during the Day 14 to 16 of estrous cycle.

Induction of CL regression by injection of prostaglandin early in the estrous cycle probably induced luteal regression and eventual ovulation of the first wave dominant follicle (Lucy et al., 1992; Macmillan and Henderson, 1983). Longer duration of estrus when prostaglandin injection was given during late luteal phase was associated with different stages of dominant follicle maturation at the time of luteolysis (Lucy et al., 1986).

In heifers, Sirois and Fortune (1988) observed a negative correlation between the size of the dominant follicle at luteolysis and the time of the surge of LH, suggesting that the time of estrus may be determined by the size of the preovulatory follicle at luteolysis. In the same way, Kastelic and Ginther (1991) reported that the time from the administration of PGF2α to ovulation is dependent on the maturity of the most recently emergent dominant follicle. The time of ovulation is therefore dependent on the size of this follicle at luteolysis, because a small dominant follicle takes longer to grow into an ovulatory follicle. Kastelic and Ginther (1991) also reported that when dominant follicle had reached the static phase, the time from treatment to ovulation was 3 d, and if a new dominant follicle emerged at the time of luteolysis, the time from treatment to ovulation was 4.5 d. Smith et al.(1998) reported that the onset of estrus was significantly and inversely related to the size of the cavity of the smallest follicle with a diameter of more than 5 mm. Several studies have reported that the stage of estrous cycle at the time of prostaglandin greatly influences the conception rate in dairy cows. Armstrong (1988) reported that the conception rate among the cows treated on Day 13 (71 %) was significantly higher when compared to the cows treated on Day 8 (46 %).

3.4.2. Effect of progesterone level on synchronized estrus

Reports are available which show that higher progesterone concentrations at the time of administration of prostaglandin are associated with delayed onset of estrus (Larson and

Ball, 1992). It has also been reported that estrus was manifested in more percentage of cows (84 %) that had high progesterone concentrations, > 3.1 ng/mL, the day of the last PGF2α injection than did cows with low progesterone levels (56 %).

The level of progesterone levels prior to ovulation following the administration of prostaglandin affect the fertility of cows in synchronized estrus. Folman et al. (1990) found that cows conceiving to AI at induced estrus had higher progesterone levels during the proceeding luteal phase than those not conceiving. However, Gyawu et al. (1991) showed that excessively long periods of high progesterone prior to insemination can suppress fertility. Folman et al. (1990) reported that the number of primiparous cows conceived following administration of prostaglandin at 14 d interval is significantly more than cows administered prostaglandin at 11 d interval due to increased level of progesterone prior to ovulation, when prostaglandin was administered at 14 d interval (Rosenberg et al., 1990).

3.4.3. Effect of different prostaglandin analogues on estrus response and fertility.

The fertility of estrus, induced with different analogues of prostaglandin was reported to be similar to that of estrus induced with prostaglandin (Martinez and Thibier, 1984; Seguin et al., 1985). However, El-Menoufy and Abdou (1989) reported that the estrus synchronization rate was higher in cows treated with cloprostenol (90 %) when compared to cows treated with prostaglandin (82%). Schams and Karg (1982) compared the luteolytic action of alfaprostol, cloprostenol, prosolvin and tiaprost in heifers and reported that there was difference among the various analogues concerning their luteolytic action on the CL. Wenzel, (1991) reported that a greater proportion of cows with unobserved estrus show luteolysis and behavioral estrus when treated with prostaglandin and fenprostalene than cows treated with cloprostenol.

3.4.4. Route and dose of PGF2α administration:

Even though, prostaglandin has been administered usually by intramuscular injection, various other routes viz. intravenous (Maurer et al., 1989; Stevens et al., 1995), subcutaneous (Brogliatti et al., 2000; Colazo et al., 2002a; Colazo et al., 2002b) and through ischiorectal fossa (Colazo et al., 2002b) have also been reported in cattle. Maurer et al 1989 found that cows treated with prostaglandin intravenously had heavier CL and less reduction in serum progesterone concentration at 24 h after treatment than cows that were treated with prostaglandin intramuscularly. They suggested that prostaglandin injected intravenously would metabolize faster, resulting in less peripheral exposure time. Sevens et al. (1995), however, reported that cloprostenol administered intravenously to nonlactating diestrus dairy cows was not found to affect the rate of luteolysis, compared to cows given cloprostenol intramuscularly.

Prostaglandin had a very short half-life and once absorbed into the blood stream, is quickly inactivated by oxidation after one passage through the lungs (Kindahl, 1980). Plasma concentrations of prostaglandin were raised to maximum level within 10 min of exogenous administration of PGF2α and it declines to pre-injection level by 90 min (Stellflug et al., 1975). Therefore, many works have been done to determine the minimum effective dose and the most appropriate route of administration of the drug. Several reports are available regarding administration of reduced dose of prostaglandin being given into various locations in the reproductive tracts namely, intravulvosubmucosal (IVSM) (Ono et al., 1982; Basurto-Kuba et al., 1984; Chauhan et al., 1986; Horta et al., 1986; Pawshe et al., 1991; Canizal et al., 1992; Dhande and Kadu, 1994; Honaramooz and Fazelie, 1995; Colazo et al., 2002b), intraovarian (Bermubez et al., 1999), deposition into cervix or vulvar lips (Galina and Arthur, 1990), intrauterine infusion (Tervit et al., 1973; Louis et al., 1974; Betteridge et al., 1977; Chatterjee et al., 1989) and injection into the uterine wall (Inskeep, 1973).

In case of IVSM route of administration of prostaglandin, many authors recommend that the injection should be given on the side ipsilateral to the CL (Rao and Venkatramaiah, 1989; Canizal et al., 1992) as, the drug reaches the ovary through a local pathway and without entering the systemic circulation (Chauhan et al., 1986; Horta et a., 1986). Whereas, Colazo et al.(2002b) found no support to this recommendation since, in cows there is no relationship between the venous drainage of the vulvar region and the ovarian arterial supply (Ginther, 1974; Ginther and Del Campo, 1974).

Though it is widely accepted that the intramuscular dose of PGF2α and its analogues for estrus synchronization is 25 mg and 500 μg respectively in cattle (Lagar, 1977), several workers attempted reduced dose of prostaglandin in dairy cows and heifers with normal or below normal estrus response and fertility (Nakahara et al., 1975; Kiracofe et al., 1985; Narayana and Honnappa, 1986; Berardinelli and Adiar, 1989; Plata et al., 1989; Garcia-Winder and Gallegos-Sanchez, 1991; Rivera et al., 1994). The stage of estrous cycle, and presumably the stage of the functional CL, affects the efficacy of a reduced dosage of PGF2α to induce luteolysis. Berardinelli and Adair, (1989) found greater CL sensitivity to a reduced dose of dinoprost during the late luteal phasein cattle.

3.4.5. Breed and Season

Use of prostaglandin for synchronization of estrus had less success in Bos indicus when compared to Bos taurus (Hardin et al., 1980 a,b; Hardin and Randel, 1982). Hansen et al. (1987) reported that Brahman heifers required higher dose of alfaprostol than Brahman cows for synchronization of estrus.

Interval to estrus after prostaglandin is affected by age and breed (Burfening et al., 1978) and season (Britt, 1979; Jaster et al., 1982). Jaster et al. (1982) recorded the influence of season in response to prostaglandin affects the estrous behavior and conception rate. They recorded a high conception rate when synchronization program was conducted during July (50 %) than in December (20 %).

3.4.6. Effect of pheromones on luteolytic action of prostaglandin.

Izard and Vandenbergh (1982) observed that pheromones from the cervical mucus of estrous cows affect the ovarian function of herdmates and thereby improve the synchrony of estrus after administration of prostaglandin.

3.4.7. Presence of bull.

Galina and Arthur (1990) reported that the presence of a bull with cows synchronized with prostaglandin markedly influenced the behavioral pattern of the herd. They found that the pattern of mounting activity of the synchronized cows was more spread out when a teaser bull in present when compared to the synchronized cows without a bull.

3.5. Factors limiting the use of prostaglandin in dairy cows

The luteolytic action of PGF2α is used considerably as a drug for estrus synchronization and controlled breeding schemes with the objective to improve the reproductive performance of dairy cows. However, a proportion of failures occur, mainly cows not exhibiting estrus within the expected time period following the injection of PGF2α (Wenzel, 1991). The reasons for the failure of luteolytic action of PGF2α are reviewed below.

3.5.1. Effect of accuracy in rectal palpation of CL

Although prostaglandin protocols are applied without screening the ovarian status, gynaecology examination by way of rectal palpation of ovary is often done to detect a mature CL before a single prostaglandin dose protocol (Wenzel, 1991).

One major reason for decrease in the success of estrus synchronization following administration of prostaglandin is due to the unreliability of CL palpation by rectal examination (Ott et al., 1986). The accuracy of rectal palpation in determining the presence or absence of mature CL has been reported by various authors (Boyd and Munro, 1979; Watson and Munro, 1980; Mortimer et al., 1983). Even though the handling serum (Vahdat et al., 1979; Fahmi et al., 1985) and plasma (Vahdat et al., 1979, 1981, 1984) samples have been shown to affect progesterone assay results, the concentration of progesterone in plasma (Boyd and Munro, 1979), Serum (Mortimer et al., 1983) or milk (Watson and Munro, 1980) was used as the standard against which palpation for the presence or absence of mature CL was judged. Ott et al. (1986) showed that there was only 77 % agreement between diagnosis of CL by experienced palpator and the progesterone concentration. Further, they reported that identification of a CL by rectal palpation was 85 % accurate and no CL was false as many times as it was true. Whereas, Seguin et al. (1978) and Dailey et al. (1986) reported palpation error up to 6 % during identification of a CL by rectal palpation. Similarly, Kelton et al. (1991) reported that the success of estrus synchronization depends on the accurate identification of a mature CL by rectal palpation.

3.5.2. Number of cows in synchronized estrus.

Another reason that affects the potential use of PGF2α in improving the pregnancy rate in the herd is due to the presence of often a very high number of cows in estrus at a given time after the administration of PGF2α for synchronized estrus, which reduces the estrus detection efficiency in the herd. (Seguin et al., 1985).

3.5.3. Level of progesterone during prostaglandin treatment.

It has been shown that there is a positive correlation between the level of progesterone in plasma (Lucy et al., 1986; Folman et al., 1990; Stevens et al., 1993) or in milk (Dailey et al., 1986) and the conception rate in PGF2α induced estrus indicating that the conception rate in cows following PGF2α injection has been positively correlated with the plasma concentration of progesterone that is reached during the days preceding the luteolysis (Chenault et al., 1976; Jaster et al., 1982; Folman et al., 1990). Birnie et al. (1997) observed that the efficacy of prostaglandin as luteolytic agent is reduced when it is administered along with GnRH.

3.5.4. Variation in duration of onset of estrus

Another limiting factor in the use of PGF2α, is the variation in the duration of onset of estrus after the injection of the drug and estrus is not being precisely synchronized. This duration of onset of estrus following the injection of PGF2α ranges from 2 to 5 d in cattle (Cooper, 1974; Lauderdale et al., 1974; Johnson, 1978; King et al., 1982; Dailey et al., 1983; Plunkett et al., 1984; Tanabe and Hann, 1984; Watts and Fuquay, 1985; Dailey et al., 1986).

When PGF2α is administered to the cows having functionally mature CL, 85 to 95 % of the cows would be in estrus within Day 7 of injection (Macmillan and Henderson, 1983; Armstrong et al, 1989; Folman et al., 1990; Rosenberg et al, 1990) and 70 to 90 % of these cows will exhibit the estrus on Day 3 to 5 after the injection of PGF2α (Ferguson and Galligan, 1993). This variation in the time of ovulation is the major obstacle, which causes substantially lower pregnancy rate per AI in timed insemination when compared to AI after a detected estrus induced by PGF2α in lactating dairy cows (Lucy et al., 1986; Stevenson et al., 1987; Archbald et al., 1992).

3.6. Characteristics of spontaneous estrus following prostaglandin treatment.

Even though, the physiological events following the injection of PGF2α or analogues of PGF2α were reported to be similar with that of naturally occurring luteolysis (Schultz, 1980; Seguin, 1980), it takes longer (23 to 25 d) time for the occurrence of spontaneous natural estrus, following the synchronization of estrus with prostaglandin in dairy cows (Howard et al., 1990a; Cardenas et al., 1991; Morbeck et al., 1991). Larson and Ball (1992) reported that if plasma progesterone levels were higher than 1 ng/mL at the time of first injection of PGF2α, the cycle subsequent to the second injection was longer (26 versus 22.6 d) than in cows with low progesterone at first injection. However in case of heifers, the mean duration for the occurrence of natural estrus following synchronized estrus was 20.6 d (Howard and Britt, 1990; Howard et al., 1990b).

There is also a report that 25 % of heifers (Stevenson et al., 1984; Tanabe and Hann, 1984) and 50 % of cows (Graves et al., 1974; Plunkett et al., 1984) fail to conceive in spontaneous natural estrus following synchronized estrus. In case of heifers, Morrell et al. (1991) found an apparent decline in fertility following repeated estrus synchronization with cloprostenol.

4. Prostaglandin based combination treatments in dairy cows.

Following the successful reports of prostaglandin and its analogues in controlling the estrous cycle in cattle, different prostaglandin based combination treatments (Figure 1) were developed for estrus synchronization program in dairy cows (Gordon, 1996).

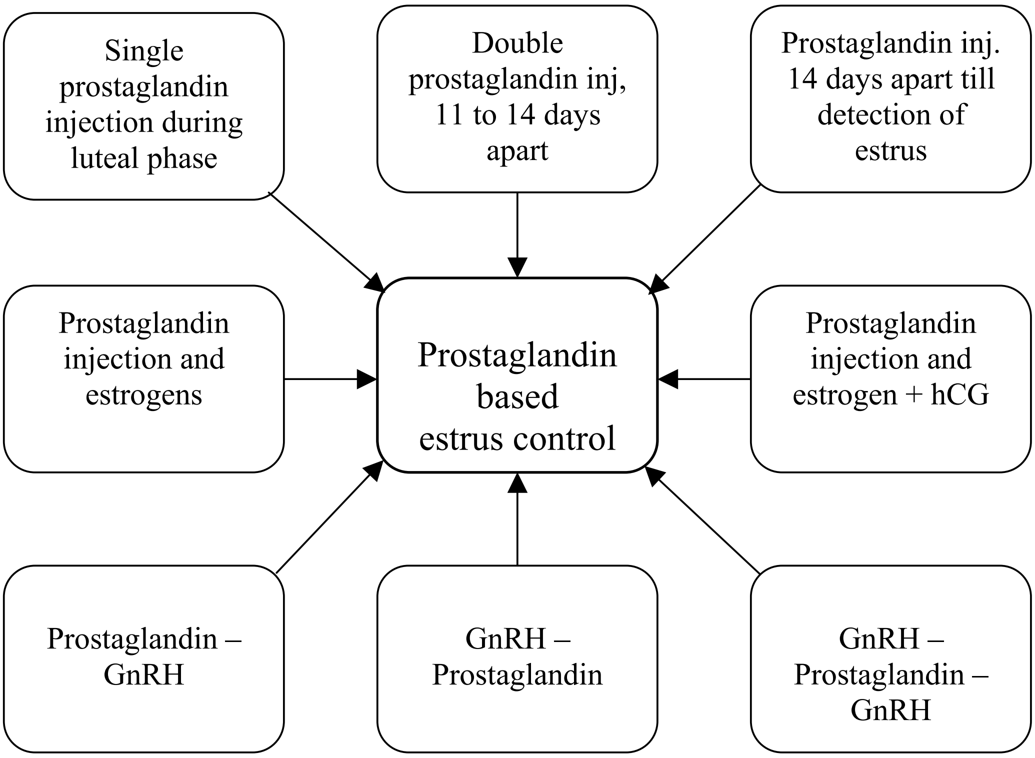

Figure 1. Prostaglandin based combination treatments employed in estrus control in the cows

4.1. Use of estrogen during prostaglandin based synchronization of estrus.

Several papers are available regarding the successful administration of estradiol benzoate following prostaglandin treatment for synchrony of ovulation (Welch et al., 1975; Peters et al., 1977; Inskeep et al., 1980) in cows and (Dailey et al., 1983) in heifers. Reports show that treatment with estradiol 24 h after induced luteolysis may be the optimum timing (Nancarrow and Radford, 1975; Ryan et al, 1995b) and that a injection of 0.5 mg estradiol benzoate (Hansel et al., 1975; O'Rourke et al., 2000) will induce a peak concentration of serum estradiol similar in magnitude to that occurring at natural estrus (Glencross and Pope, 1981). Evans et al., (2003) reported that administration of 0.5 mg of ODB 24 h after prostaglandin leads to a predicable onset of estrus, LH surge and ovulation, regardless of the stage of follicle development at treatment. Dailey et al. (1986) reported that tighter synchrony of estrus can be achieved by administration of 400 mcg of estradiol benzoate 40 to 48 h after prostaglandin treatment in dairy cows. They also observed that estrogen treatment tends to increase a greater proportion of synchronized cows to estrus on Day 3 (66.9 %) than those not receiving estrogen (48.2 %), without affecting the conception rate. However in heifers, Dailey et al., (1983) reported that administration of estradiol benzoate 48 h after prostaglandin treatment was not found to improve the synchronization and conception rates. Similarly Davis et al., (1987) found no improvement in conception rates following fixed timed insemination in beef cows and heifers treated with prostaglandin-estradiol benzoate regime when compared with cattle treated only with prostaglandin.

4.2. Use of estrogen and hCG in prostaglandin based regimes

In cows, estrogens are known to induce a preovulatory-like LH surge, ovulation (Lammoglia et al., 1998) and luteolytic activity during the luteal phase (Salfen et al., 1999). These effects could justify the inclusion of estradiol in the different synchronization regimes. Indeed, as noted above, progestogen- estrogen combinations are widely used. While synchronizing estrus using prostaglandins, ovulation was successfully synchronized by administering estradiol benzoate following prostaglandin treatment in cows (Welch et al., 1975) and in heifers (Dailey et al., 1983). A tighter synchrony of estrus with no effect on the conception rate was reported after treating dairy cows with 400 mcg of estradiol benzoate 40 to 48 h after prostaglandin treatment (Dailey et al., 1986). An estrogen-prostaglandin combination protocol for synchronization of estrus was also found to increase the percentage of cows in estrus (Figueroa et al., 1988).

The hormone hCG induces potent LH activity in ovarian cells which can even lead to ovulation throughout the estrous cycle (Price and Webb, 1989). The simultaneous administration of hCG and estradiol benzoate 12 h after treatment with prostaglandins in dairy cows and heifers with mature CL has been reported to shorten the mean time to onset of estrus and increase the precision of synchrony in ovulation. Using this protocol, comparable pregnancy rates were achieved following fixed-time insemination to those recorded when cows were treated with prostaglandin alone (López-Gatius, 1989, 2000b) or inseminated at natural estrus (López-Gatius and Vega-Prieto, 1990; López-Gatius, 2000a).

López-Gatius, (1989) reported that the high degree of estrus synchrony following Prostaglandin-hCG and estradiol regime may be due to an affinity of the luteolytic effects of exogenous estrogens (Greenstein et al., 1958; Wiltbank et al., 1961; Brunner et al., 1969; Lewis and Wassen, 1974; Eley et al., 1979) with the luteolytic effect of prostaglandin or an affinity of the response of exogenous estradiol and the ovulatory surges of LH (Schillo et al., 1983; Jacobs et al., 1988; Nanda et al., 1988) with a high degree of luteinizing activity of hCG.

5. GnRH treatment estrus synchronization protocol in postpartum cows.

5.1. Effect of GnRH on follicular dynamics.

In cycling cows, administration of GnRH or a derivative induces a gonadotropin surge (Foster et al., 1980; Chenault et al., 1990; Evans and Rawlings, 1994) with peak LH within 2 to 3 h (Williams et al., 1982) and alters the pattern of follicle growth (Kesler et al., 1980; Thatcher et al., 1989; Wolfenson et al., 1994). Administration of GnRH induces a LH surge with similar maximum LH concentrations (20.6 ± 2.8 ng / mL) (McDougall et al., 1995) but with approximately half the duration (Chenault et al., 1990), when compared to the endogenous LH release during the normal estrous cycle at the time of ovulation (Chenault et al., 1975; Rahe et al., 1980). A single injection of GnRH or an agonist is sufficient to induce ovulation or atresia of a dominant follicle (Garverick et al., 1980; Crowe et al., 1993; Twagiramungu et al., 1995a). Several reports demonstrated that growing follicles greater than 10 mm in diameter ovulate after GnRH injection (Prescott et al., 1992; Pursley et al., 1995; Silcox et al., 1995; Martinez et al., 1999).

In cattle, administration of GnRH during the early or mid luteal phase causes an alteration of follicular distribution in the ovary by increasing the number of medium sized follicle and decreasing the number of large follicles by inducing luteinization and or atresia (McNatty et al., 1981; Thatcher et al., 1989; Guilbault et al., 1990). GnRH administered on Day 11 to 13 of estrous cycle alters the ovarian follicular dynamics (Skaggs et al., 1986) since the dominant follicle either luteinizes (Thatcher et al., 1989) or develops into a secondary CL following ovulation (Stevenson et al., 1993). Wolfenson et al. (1994) studied the dynamics of follicular development by ultrasonography in cows following administration of a single dose of GnRH in the mid luteal phase (Day 12) of the estrous cycle. They reported the preovulatory follicles in cows following the injection of GnRH during the luteal phase were more homogeneous (belonging to the same follicular wave), more estrogen-active, probably due to preovulatory follicles being recruited and selected close to the time of estrus, and more dominant.

GnRH induced ovulation or atresia of dominant follicle is followed by a new wave emergence within 3 to 4 d of treatment at any stage of estrous cycle (Twagiramungu et al., 1995a). Administration of GnRH induces a FSH increase at any stage of the estrous cycle (Ryan et al., 1998). Thus, in cows treated with GnRH after the selection of a dominant follicle, gonadotropin surge is followed by a transient FSH increase, that is associated with the emergence of a new follicle wave. When GnRH treatment is applied before the selection of the dominant follicle, follicular growth is not affected (Ryan et al., 1998).

5.2. Response of GnRH at different stage of estrous cycle in postpartum cows.

Vasconcelos et al. (1999) recorded low ovulation rate (23 %) when cows are treated with GnRH during the early part of estrous cycle. Low ovulation rate following GnRH injection given at the early stage of estrous cycle have been related to the fact that protein (Bodensteiner et al., 1996) or mRNA (Xu et al., 1995; Bao et al., 1997) for LH receptor are not expressed in the granulose cells of growing follicles during first 2 d of the follicular wave. After the follicular deviation (Day 4 to 5 of estrous cycle) and prior to loss of follicular dominance, the dominant follicle has been found to express LH receptor (Bodensteiner et al., 1996; Bao et al., 1997) and all the follicles have ovulatory capacity leading to higher ovulation rate (96 %), when GnRH administered during 5 to 9 d of estrous cycle (Vasconcelos et al., 1999). They also observed low ovulation rate (54 %) when GnRH is injected near mid-cycle and during this period, the ovulation rate following GnRH injection depends on the presence or loss of functional dominance in the largest follicle of the first follicular wave. During near mid cycle, there is loss the functional dominance in the most of the largest follicles of the first follicular wave, increased serum FSH concentrations, and emergence of a new follicular wave (Ginther et al., 1996). But it has been reported that the day of the estrous cycle for loss of functional dominance is variable and is altered by many factors like nutrition (Murphy et al., 1991), heat stress (Wehrman et al., 1993) and growth hormone treatment (Lucy et al., 1994; Kirby et al., 1997).

When GnRH injection is given during the late estrous cycle, the percentage of cows that ovulate depends upon whether a new follicular wave is occurring at that time and this, in turn, is probably a function of whether a cow has 2 or 3 follicular waves during the estrous cycle (Vasconcelos et al., 1997). Pursley et al. (1996), recorded 100 % ovulation rate when GnRH administered in the late estrous cycle in a herd of cows where only 2 follicular waves in over 90 % of estrous cycle monitored. Whereas Vasconcelos et al. (1999) found 77 % ovulation rate when GnRH given in the late estrous cycle. The latter authors reported that the low ovulation rate following GnRH at late luteal phase might be probably due to increased frequency of cows with 3 follicular waves.

5.3. GnRH –Prostaglandin protocol in dairy cattle

In lactating dairy cows, application of synchronization of estrus with PGF2α protocols is very much limited because of the presence of anoestrus cows (Stevenson and Pursley, 1994), a large variation in time from regression of the CL to expression of estrus (Stevenson et al., 1987; Stevenson et al., 1989; Stevenson and Pursley, 1994) and abnormal patterns of ovarian activity in post partum dairy cows (Bulman and Wood, 1980). This variation in time to estrus is due to differences in the developmental stage of the preovulatory follicle at the time of PGF2α injection (Fortune et al., 1991) and is related to the rate of progesterone decrease to basal level (King et al., 1982). In double injections at 14 d apart regime, the CL of at least 67 % of randomly cycling cows on Day 7 to 20 of their cycle undergo luteolysis either spontaneously (Day 18 to 20) or in response to prostaglandin (Day 7 to 17). Therefore, this first group of cows should be on Day 9 to 14 of their estrous cycles when the second PGF2α is given 14 d later. The second group of remaining cows (33 %; Day 0 to 6 of their cycle) should be on Day 14 to 20 of their cycle when second PGF2α is given (Lucy et al., 1992). Few cows in first group will have a mature first wave dominant follicle, but the majority of the first group and all of the second group will have a maturing second wave dominant follicle that is capable of ovulating in response to GnRH induced LH release at some interval after luteal regression is induced by a second PGF2α injection. (Lucy et al., 1992). To improve the estrus synchrony exogenous GnRH, which controls the developmental stage of the preovulatory follicle has been included with prostaglandin for synchronization of estrus in dairy cows. (Son and Larson. 1994; Stevenson and Pursley, 1994; Thatcher et al., 2001).

The random administration of GnRH during the estrous cycle results in LH release (Chenault et al., 1990), causes ovulation or luteinization of large follicles present in the ovary, synchronizes the recruitment of a new follicular wave (Thatcher et al., 1989; Martinez et al., 2000a), and equalizes follicle development waves (Thatcher et al., 1989; Twagiramungu et al., 1992; Wolfenson et al., 1994; Schmitt et al., 1996a,c). Subsequent administration of PGF2α induces the regression of an original or GnRH-induced CL, and allows final maturation of the synchronized dominant follicle (Schmitt et al., 1996b). Further, there is no apparent detrimental effect of GnRH on the responsiveness of GnRH-induced CL or spontaneous CL to prostaglandin (Twagiramungu et al., 1995a).

5.4. Simultaneous administration of GnRH and prostaglandin in dairy cows

Stevens et al. (1993) reported that administration of GnRH and prostaglandin simultaneously on Day 8 or 10 of estrous cycle does not improve the synchrony of estrus and ovulation (luteolysis in only 6 of 16 animals) because GnRH disrupts follicular dynamics and induces premature ovulation or delays the normal return to estrus. Birnie et al. (1997) treated heifers with GnRH injections every 24 or 48 h from Day 3 until Day 17 of estrous cycle and administered prostaglandin on Day 13 of estrous cycle to study the luteal response of GnRH treated animals to a physiological dose of prostaglandin. They observed the luteolytic activity by using ultrasonography only in seven of 16 animals in GnRH treated group. Birnie et al (1997) reported that the luteolytic activity PGF2α analogue is reduced when it is administered in combination with the GnRH agonist. They reported that reduced luteolytic effect of prostaglandin when given simultaneously with GnRH may be due to a luteotropic protection of GnRH on the CL, thus preventing the usual cascade of oxytocin stimulation and progesterone inhibition that occurs until completion of luteolysis.

5.5. GnRH – Prostaglandin protocol on reproductive performance in dairy cows

Several reports (Twagiramungu et al., 1992; Thatcher et al., 1989; Stevenson et al., 1999) have described a higher rate of estrus synchronization when GnRH is administered 6 or 7 d before PGF2α (80%) compared to prostaglandin alone (50 to 60%). However, LeBlanc et al. (1998) reported no advantage of adding GnRH on Day 7 of a synchronization program based on double prostaglandin treatment given at a 14 d interval. Similarly, Stevenson et al. (1996) described a decreased conception rate (48.1%) when GnRH was administered between two PGF2α doses given 14 d apart compared to not including GnRH in the protocol (63.5%).

Pursley et al. (1995) observed a mean reduction of 27 d to first AI with a voluntary waiting period of 50 d after a GnRH-PGF2α regimen. The same regime was found to fail to induce estrus in some cows due to incomplete luteolysis following prostaglandin treatment (Twagiramungu et al., 1994) or because of differences in pituitary LH release at the time of treatment (De Rensis et al., 1999).

5.6. GnRH – Prostaglandin – GnRH combination

To synchronize the ovulation time within a short time period to enable timed insemination in the GnRH – prostaglandin regime, an additional dose of GnRH was included at 32 to 48 h after the prostaglandin treatment. This Ovsynch protocol could have a major impact on managing reproduction of lactating dairy cattle, since it could permit AI to be performed at a known time of ovulation and would eliminate the need for the detection of estrus (Pursley et al., 1995).

5.6.1. Control of ovulation by the second dose of GnRH during preovulatory period.

To synchronize the LH surge and the ovulation in GnRH and PGF2α treated animals, a second injection of 100 μg GnRH was administered at 0 (Pursley et al., 1995), 24 (Pursley et al., 1995; Thatcher et al., 1996), 48 (Pursley et al., 1995), 54 (Twagiramungu et al., 1995b) and 48 to 72 (Peters et al., 1999) h after prostaglandin treatment. A second dose of GnRH given 48 h after PGF2α injection improves the precision of ovulation over an 8 h period from 24 to 32 h after this second GnRH dose (Pursley et al., 1995). The success of this addition to the standard combined GnRH-prostaglandin regime in dairy cattle gave rise to the recently developed Ovsynch or timed artificial insemination (TAI) protocol, which allows successful fixed-time AI without the need for estrus detection (Pursley et al., 1995).

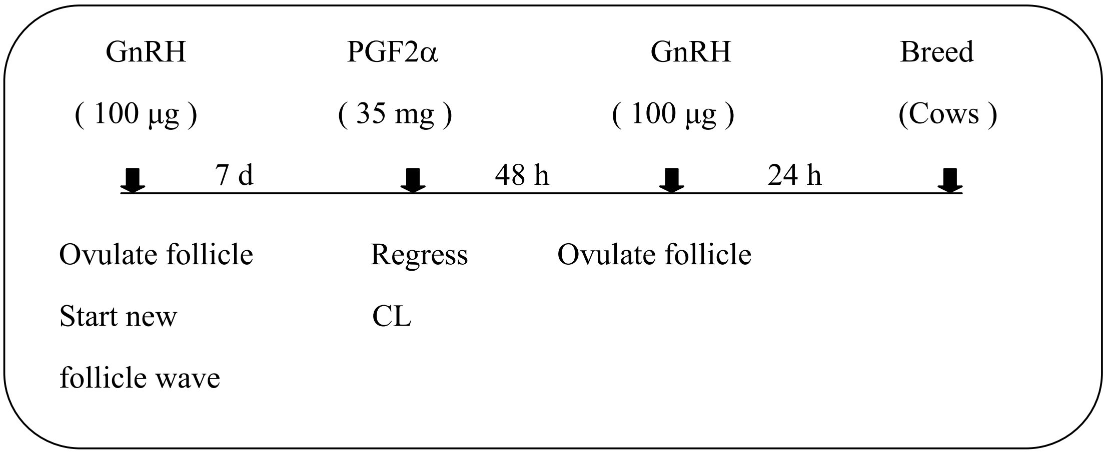

Figure 2. Timing and purpose of hormones employed to synchronize ovulation (Ovsynch protocol) in the lactating dairy cows (from Pursley et al., 1995).

In the Ovsynch program (Figure 2), 100 µg of GnRH are given at random during the estrous cycle, followed by 25 mg of PGF2α on Day 7 and a second dose of 100 µg GnRH 48 h later (Pursley et al., 1995; Pursley et al., 1997b). Ovulation is synchronized because the preovulatory follicles are at a similar stage in development and is responsive to LH at the time of the second GnRH treatment. This program coordinates follicular recruitment, CL regression and time of ovulation and permits fixed time AI 16 h after the second GnRH dose is administered. Thus by synchronizing ovulation, reproduction in lactating dairy cows can be effectively managed without the need for estrus detection. Pursley et al. (1998) concluded that AI performed close to 16 h after the second dose of GnRH in the Ovsynch protocol seems to be optimal, though pregnancy rates per AI and calving rates are comparable to rates achieved following AI performed 0 to 24 h after the second GnRH dose. Recently, the Ovsynch protocol has been slightly modified such that the second GnRH dose is given 36 h instead of 48 h after prostaglandin treatment (Nebel and Jobst, 1998). Fricke et al. (1998) and Yamada et al. (2002) reported that the reproductive performance in dairy cattle is not affected when the GnRH dose is reduced to half (50 µg instead of 100 µg) in the Ovsynch protocol. Pursley et al. (1998) concluded that AI near 16 hr after the LH surge seems to the optimal time in Ovsynch protocol, even though pregnancy rate per AI and calving rates following AI done from 0 to 24 h after second GnRH injection are similar.

5.6.2. Influence of the stage of estrous cycle at the time of initiation of Ovsynch protocol

It has been demonstrated that the success of Ovsynch program is influenced by the number of follicular waves or length of the follicular wave (Pursley et al., 1997b) and the stage of estrous cycle when the first GnRH is administered (Vasconcelos et al., 1997; Vasconcelos et al., 1999; Moreira et al., 2000a).

The initiation of the Ovsynch treatment at metaphase stage of estrous cycle may lead to failure of synchronization of a new follicular wave by the first GnRH since the follicle present at the time of first GnRH will be small and fail to ovulate. If dominant follicle is too small at the time of GnRH injection, it does not respond to GnRH induced LH surge because of the lack of LH receptors on the granulose cells (Twagiramungu et al., 1995a; Xu et al., 1995). Consequently the dominant follicle will undergo early stage of atresia at the time of second GnRH. Thus the poor quality of the preovulatory follicle and subsequent development of the aged oocyte may affect the pregnancy rate in dairy cattle (Wishart, 1977; Mihm et al., 1994). In addition, a markedly lower fertility was also found due to ovulation of a large persistent follicle in cows under treatments that provide only low progestin concentrations prior to ovulation (Savio et al., 1993 and Stock and Forture, 1993). Low fertility in these persistent follicles are attributed to the premature oocyte activation (Revah and Butler, 1996) and effect of low progesterone on subsequent uterine function (Shaham-Albalancy et al., 1997).

When Ovsynch program is initiated at late luteal phase of the estrous cycle, the animal may undergo premature CL regression and estrus is observed before the second injection of GnRH. Since the normal CL regression starts Day 16 of estrous cycle (Ginther et al., 1989a), the CL will under go spontaneous regression 3.2 d before the injection of PGF2α by the normal endogenous release of endometrial PGF2α. Vasconcelos et al., (1999) observed that ovulation in response to the first injection of GnRH is important for the success of Ovsynch protocol, particularly for the cows in the late estrous cycle.

During the prophase of the estrous cycle, initiation of Ovsynch leads to incomplete regression of induced accessory CL by PGF2α. CL that developed under a low progesterone environment may be on borderline of being responsive to an injection of prostaglandin (Watts and Fuquay, 1985). Incomplete regression of CL following PGF2α in Ovsynch is associated with a lower pregnancy rate (Moreira et al., 2000b).

Moreira et al. (2000a) concluded that the early luteal stage of the estrous cycle (Day 5 to 12) was the optimal period for initiating the Ovsynch program. Similarly, Vasconcelos et al. (1997), also recorded higher pregnancy rate in cows initiated Ovsynch protocol during early luteal phase when compared to the cows initiated the treatment during first 3 d or after Day 13 of estrous cycle. These findings are however inconsistent with those of Keister et al.

(1999), who noted similar reproductive performance in dairy cattle whether Ovsynch treatment was initiated at random or on Day 7 of estrous cycle.

Based on the reports that the luteal phase was the optimal time of Ovsynch protocol onset in terms of conception rates, Moreira et al. (2001) presynchronized cows using two prostaglandin doses given 14 d apart to initiate the Ovsynch protocol at the targeted early luteal phase. Presynchronization was found to increase the pregnancy rate in cyclic lactating dairy cows. Similarly, pregnancy rates in dairy cows were improved when Ovsynch was started on Day 12 (Cartmill et al., 2001a) or Day 14 (Jordan et al., 2002) after prostaglandin administration, since most cows would be in early diestrus before the beginning of the Ovsynch protocol. Bartolome et al. (2002) obtained similar pregnancy rates between cows with and cows without CL following Ovsynch protocol, by presynchronizing cows with palpable CL using prostaglandin 14 d before initiation of Ovsynch and for cows without palpable CL, using GnRH 8 d before beginning of Ovsynch. However, no beneficial effects were shown by presynchronization prior to Ovsynch in anestrous cows, given their lack of prostaglandin responsive CL (Moreira et al., 2001).

5.6.3. Aspects of reproductive performance following Ovsynch program in dairy cattle

Although many workers (Keister et al., 1999; Burke et al., 1996; Pursley et al., 1997a; Mialot et al., 1999; Cartmill et al., 2001b) have reported increased pregnancy rates in cows subjected to Ovsynch treatment, this increase has not been paralleled by conception rates because of the greater number of cows inseminated after Ovsynch treatment (Stevenson et al., 1996, 1999). When Burke et al. (1996) compared the effectiveness of timed AI following Ovsynch protocol versus AI at detected estrus after Ovsynch without administering the second GnRH dose in multiparous animals, they recorded higher conception rates in cows undergoing AI at detected estrus, but pregnancy rates were similar in both groups. These authors also noticed a mean reduction to first AI in the timed AI program of 9.7 d, compared to AI at detected estrus following a 60 d voluntary waiting period. DeJarnette et al. (2001) claim that pregnancy rates in the Ovsynch protocol can be maximized by improving estrus detection, since 20% of the cows display estrus outside the optimal time period for conception by TAI.

Timed AI following the Ovsynch protocol is advocated by several authors (Burke et al., 1996; Yamada et al., 1999; Momcilovic et al., 1998) as an effective tool for improving reproductive management in dairy cows, since it avoids the need for estrus detection.

Whereas in heifers, Pursley et al. (1997b) observed a decrease in pregnancy rate using Ovsynch when compared to pregnancy rates following synchronization of estrus with three consecutive prostaglandin treatment at 14 d intervals. They also recorded a lower ovulation rate (54 %) in heifers than lactating dairy cows (85 %) following the first injection of GnRH possibly because of the inconsistent follicular wave patterns. The decreased success in Ovsynch program in heifers is attributed to the lack of follicular synchrony due to low ovulatory response following the first dose of GnRH injection.

5.6.4. Influence of various factors on reproductive performance following Ovsynch.

5.6.4.1. Influence of high milk yield.

Vasconcelos et al., (1999) observed that high milk production is positively correlated with increased follicular size, leading to lower fertility following Ovsynch program. They reported that high milk production leads to reduce serum progesterone concentration due to increased metabolism of progesterone and increase in follicular size due to increased in LH pulse frequency (Adams et al., 1992; Bergfelt et al., 1991; Roberson et al., 1989).

5.6.4.2. Influence of progesterone level at the time of PGF2α treatment.

Pursley et al. (1995) showed that the pregnancy rate following Ovsynch treatment is similar in cows regardless of concentration of progesterone level at the time of PGF2α injection. Whereas, heifers with low progesterone concentration at the time of PGF2α injection had a lower pregnancy rate per AI than heifers with high progesterone concentration level at the time of PGF2α injection. However, Burke et al. (1996) reported that conception rate and pregnancy rates following Ovsynch program is influenced positively by the plasma concentration of progesterone at 65 d postpartum.

5.6.4.3. Influence of stage of lactation at the time of treatment

The stage of lactation of multiparous cows was found to affect the pregnancy rates following Ovsynch treatment. The pregnancy rate in Ovsynch protocol was lower in cows during Day 60 to 75 postpartum than cows in greater than Day 76 postpartum indicating that the voluntary waiting period (VWP) of at least 75 d postpartum is required for increasing the pregnancy rate in Ovsynch treatment (Pursley et al., 1997b).

5.6.4.4. Influence of body condition of cows at the time of treatment

While Momcilovic et al. (1998) recorded no effect of body condition score and the lactation number on the reproductive characteristics, Burke et al. (1996) and Mattos et al. (2001) found positive influence of body condition score on the pregnancy rate following Ovsynch treatment.

5.6.4.5. Influence of heat stress during Ovsynch treatment

When cows were exposed to high environmental temperature, reduction in estrus detection rate and poor expression of estrus (Thatcher, 1974; Thatcher and Collier, 1986) due to reduced plasma estradiol concentration during proestrus (de la Sota et al., 1993; Wilson et al., 1998) was reported. Heat stress was not found to affect the pregnancy rate at early stage of pregnancy ($\leq$ 30 d of pregnancy diagnosis) following Ovsynch treatment, as timed insemination was independent of either expression of estrus or detection of estrus (de la Sota et al., 1998; Cartmill et al., 2001b). However, no significant difference in pregnancy rates was recorded when pregnancy diagnosis was performed during 40 to 50 d postinsemination due to higher embryonic death during heat stress in cows treated with Ovsynch protocol (Cartmill et al., 2001b).

6. Progesterone or progestogen treatments in postpartum dairy cattle.

6.1. Advantages in the use of progestogens in synchronization of estrus in dairy cows.

One of the major limitations in the use of prostaglandins to synchronize estrus in dairy cows is the failure of the drug in anestrous or noncyclic cows (Stevenson and Pursley, 1994). Although progesterone has been scantly used in dairy cows, there is a long experience in beef cattle (Odde, 1990). Progestogens have the advantage that, besides improving estrus synchronization, they also induce estrus and ovulation to an acceptable percentage in anestrous cows (Smith and Kaltenbach, 1990; Fike et al., 1997). Several works have shown that the estrous cycle in cows can be controlled by prolonging the luteal phase or establishing an artificial luteal phase by the administration of exogenous progesterone or synthetic progestogens (Odde, 1990; Larson and Ball, 1992) as progesterone suppresses estrus and ovulation by inhibiting the release of luteinizing hormone, impeding the final maturation of follicles (Peters, 1986).

6.2. Methods of administration of progesterone or synthetic progestogens

The earliest method of administrating progestogen for synchronization of estrus was by daily injections of progesterone (Christian and Casida, 1948; Ulberg et al., 1951). Subsequently, oral administration of melengestrol acetate (MGA) or medroxyprogesterone acetate (MPA) (Hansel and Malven, 1960; Hansel, 1961; Hansel et al., 1961; Zimbelman et al., 1970) and progesterone applied via intravaginal using sponge pessaries (Sreenan, 1975; Sreenan and Mulvehill, 1975) were successfully attempted to synchronize estrus in cattle (Figure 3). Of late, progesterone or synthetic progestogens are either administered by intravaginal devices like PRID (progesterone releasing intravaginal device) (Mauer et al., 1975; Roche, 1976c) and CIDR (controlled intravaginal drug release) (Macmillan and Peterson, 1993), or subcutaneous ear implants (Wishart and Young, 1974; Wiltbank and Gonalez-Padilla, 1975). Hunter (1980) reported that the absence of a depot of progesterone or synthetic progestogen upon removal of these implants or device may have offered some advantage in obtaining close synchronization.

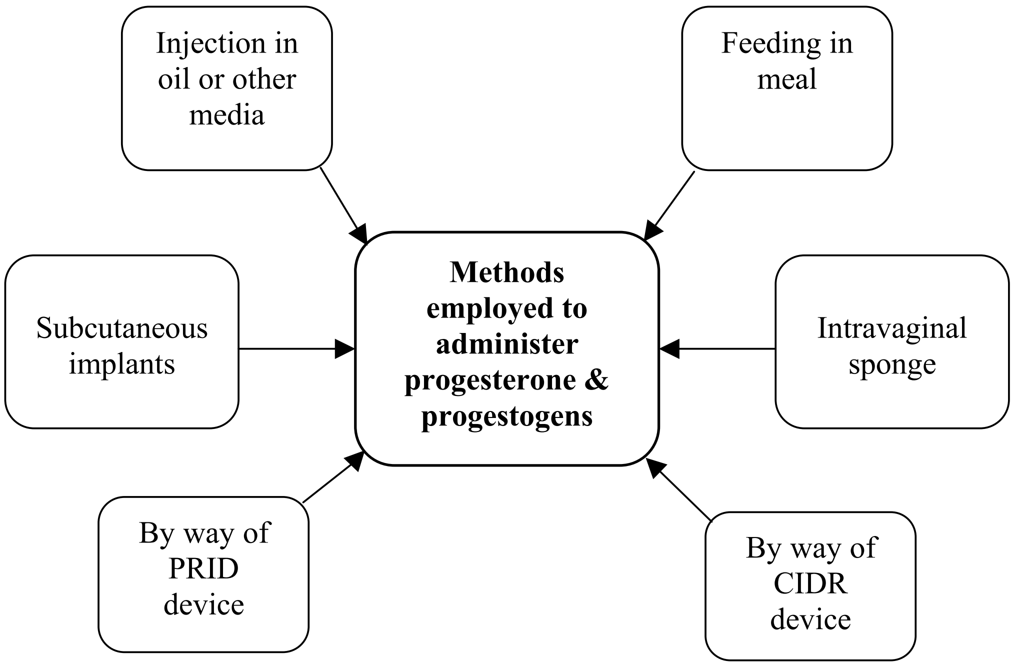

Figure 3. Methods employed in administering progestogens and progesterone in estrus synchronization in cattle (from Gordon, 1996).

Long-term progesterone treatment (14 to 16 d) leads to reduced fertility, probably due to development of persistent follicles and reduced oocyte competence (Savio et al., 1993; Revah and Butler, 1996). Progesterone administration in the absence of CL, results in the development of persistent follicles (Cupp et al., 1992) because of an increased pulse frequency of LH, similar to the pulse frequency of LH during the follicular phase of estrous cycle (Roberson et al., 1989; Kojima et al., 1992). Oocytes ovulated from the persistent follicle undergo premature resumption of meiosis and this reduced fertility could be due to asynchronous nuclear and cytoplasmic maturation of the oocytes (Mihm et al., 1994). Further, the retardation of the embryonic development by Day 6 after mating in cows that ovulated a persistent follicle could be due to increased plasma estradiol altered oocyte maturation or oviductal function (Ahmad et al., 1995).

However, Anderson and Day (1994) and McDowell et al. (1996) reported that the fertility could be improved with short-term progesterone treatment to induce regression of persistent ovarian follicles. Normal fertility was registered following short-term treatments (7 to 9 d) but with a decreased synchrony of estrus (Roche, 1974a).

6.3. Factors influencing reproductive performance following progesterone treatments

Fertility following synchronization of estrus with progesterone treatment is influenced by the blood progesterone concentration during the luteal phase before the treatment (Folman et al., 1973, 1990), by the presence or absence of CL during the progesterone treatment (Richards et al., 1990; Sanchez et al., 1993; Smith and Stevenson, 1995) and depends on the timing of various progesterone treatments on follicular dynamics during the estrous cycle (Adams et al., 1992; Custer et al., 1994).

The type of progestogens preceding estrus was also reported to alter the fertility in cows. Exogenous natural progesterone delivered via PRID resulted in greater fertility than using norgestomet, a synthetic progestin, in cows without a functional CL (Smith and Stevenson, 1995). Tjondronegoro et al., 1987 observed good synchronization rate with better synchrony of estrus following treatment with PRID than CIDR treatment in dairy cows. However, they recorded similar first service conception and pregnancy rates in the two groups of treatment. Van Niekerk et al. (1970) reported that the stage of the estrous cycle at beginning of the progesterone treatment modifies the pregnancy rates in induced estrus. In an experiment, they observed that fertility was normal when the treatment was initiated early in the estrous cycle, but reduced when initiated after Day 11 of the estrous due to development of a large abnormal follicle (Van Niekerk and Belonje, 1970). Larson and Ball (1992) concluded that incorporating a luteolytic agent 1 d before or at the time of termination of short term progesterone treatment is essential to obtain both efficient estrus synchrony and normal fertility.

7. Progesterone based combination treatments in dairy cows.

7.1. Progesterone and estrogen combinations in estrus synchronization in dairy cows

A disadvantage in the sole use of progesterone treatment in cyclic cows for estrus synchronization is the low fertility rates of synchronized estrus (Beal et al., 1988; Odde, 1990; Larson and Ball, 1992) due to extension of the life span of the dominant follicle and ovulation of subfertile oocytes (Ahmad et al., 1995; Kinder et al., 1996). Several works have recommended that the problem of persistent follicle can be avoided by an injection of estradiol at the onset of progestogen treatment (Bo et al., 1995b; Burke et al., 1998; Fike et al., 1997). Exogenous estradiol treatment suppresses the growth of the dominant follicle by suppressing gonadotropin secretion and the effect is most consistent when combined with progesterone (Price and Webb, 1988; Bolt et al., 1990; Bo et al., 2000). Termination of follicular wave results in emergence of a new follicular wave 3 to 5 d later (Bo et al., 1994a, 1995a; Martinez et al., 1997) to ensure presence of a new growing dominant follicle at the termination of progestin treatment (Twagiramungu et al., 1995a; Garcia and Salaheddine, 2001).

Evidence regarding the effect of exogenous estrogens on follicular dynamics and / or synchronization of estrus was reported in which estradiol benzoate (Macmillan et al., 1993), estradiol valerate (Bo et al., 1991; Bo et al., 1993), estradiol cypionate (Thundathil et al., 1997) or estradiol 17β (Bo et al., 1994b; Tribulo et al., 1995) was given to cows during or a few days after administration of a progestogen ear implant or a progesterone intravaginal device.

Based on the usefulness of a luteolytic agent in progesterone based treatment, estrus synchronization program has been developed using a combination of progestogen and exogenous estrogens (Wishart and Young, 1974; Spitzer et al., 1976; Favero et al., 1993). This combination treatment was extensively practiced in beef cattle (Odde, 1990), but several reports are also available in dairy cows (Gyawu and Pope, 1983; Larson and Ball, 1992). Progestogen substances are delivered in the form of an intravaginal sponge pessaries or solid phase delivery device, which provides a continuous supply of Progestogen. Such devices are used to deliver the hormone either intravaginally or via ear implants. The intravaginal sponges were generally impregnated with 3 g of the natural steroid or 200 mg fluorogestone acetate (Wiltbank and Gonzalez-Padilla, 1975).

Progesterone delivery devices either intravaginally viz. PRID (Roche, 1976b; Roche and Ireland, 1981) and CIDR (Macmillan and Peterson, 1993; Martinez et al., 2000b; Rhodes et al., 2001a,b), or by norgestomet ear implants (Spitzer et al., 1978; Pratt et al., 1991;Tregaskes et al., 1994) have been used extensively for synchronization of estrus in cows.

7.1.1. Recently used progesterone delivery devices in estrus synchronization.

7.1.1.1. Progesterone releasing intravaginal devices

For estrus synchronization in cattle, two intravaginal devices were commonly used for administration of progestogens. 1. Progesterone releasing intravaginal device (PRID): It is a metal spiral coated with progesterone- impregnated silicone elastomer and capable of releasing physiologically effective amounts of progesterone over a period of 2 to 3 weeks, containing 1.55 g of progesterone along with 10 mg of estradiol benzoate in an attached capsule (Mauer et al., 1975). 2. Controlled internal drug release device (CIDR): It is a Y – shaped nylon device about 15 cm long which is covered with a progesterone impregnated silicone elastomer, containing 1.9 g progesterone (Macmillan and Peterson, 1993).

One of the disadvantages in the use of the intravaginal devices is the loss of the device during the synchronization program (Roche, 1976 b, c). Broadbent et al. (1993) reported that CIDR was found to have a better retention rate than PRID in both dairy cows and heifers. Ryan (1994) also observed that the loss rate of the CIDR (3.5 %) was less when compared to the PRID whereas, Vargas et al. (1994) reported a very low retention rate of CIDR (85 %) in Holstein heifers. Broadbent et al. (1991) however, concluded that the loss rates should not exceed 5 % for both PRID and CIDR, if they were inserted correctly into the vaginal passage.

7.1.1.2. Norgestomet ear implants

Recently, smaller ear implants are employed effectively in synchronization of estrus in cattle (Tregaskes et al., 1994; Kastelic et al., 1999). The implant impregnated with norgestomet is implanted below the skin on the outer surface of the ear and, is accompanied by an intramuscular injection of estradiol and norgestomet.

The actions of norgestomet occurs through its binding to progesterone receptors in target tissues, and more effectively than progesterone itself Moffatt et al. (1993). It has been observed that the action of exogenous estradiol on suppression of the dominant follicle during the progesterone treatment was more consistent with progesterone implants (Bo et al., 1994a).

Sanchez et al. (1993) reported that the conception rate following norgestomet implant removal was greater in cows with a CL than those without CL. They suggested that higher estradiol concentration during the treatment period might have contributed to the reduced fertility in cows without CL.

While, Wiltbank and Gonzalez-Padilla (1975) and Miksh et al. (1978) reported high conception rates following synchro-Mate-B treatment in anestrous cows, Brink and Kiracofe (1988) recorded a low conception rate of 30 % following synchromate-B in anestrous cows and heifers. The low conception rate was attributed to luteal dysfunction (King et al., 1986; Favero et al., 1988) due to insufficient LH production following implant removal (Hixon et al., 1981).

Brink and Kiracofe (1988) found that the stage of estrous cycle at the onset of treatment influences the conception rate. They recorded a high conception rate (47 %) for heifers that were on Day 11 or less of the estrous cycle when compared to heifers (37 %) that were on Day 12 or greater of the estrous cycle at the onset of synchro-Mate-B insertion. The reduced conception rate, when the treatment was initiated at later stage of estrous cycle may be due to long term progesterone exposure (Odde et al., 1990).

An interesting report by McGuire et al. (1990) showed that synchro-Mate-B induced behavioral estrus in ovariectomied beef cows and heifers suggesting that ovaries were not necessary for an estrous response.

7.1.2. Administration of eCG during progesterone and estrogen regime

Treatment with eCG at progestogen implant removal was often recommended, especially if a high proportion of cattle are anestrus (Odde, 1990; Tregaskes et al., 1994; Humblot et al., 1996).

Kastelic et al. (1999) synchronized cattle with norgestomet silicone ear implant with 500 IU of eCG at the time of implant removal and observed that synchronization rate, synchrony of estrus and ovulation and pregnancy rates were better in cattle treated with norgestomet with eCG as when compared with those given 2 injections of cloprostenol, 11 d apart. Similarly, Fitzpatrick and Finlay. (1993) recorded a better pregnancy rate (46 %) by fixed time insemination at 48 and 60 h following norgestomet and eCG regime in Bos indicus heifers. In this regime they administered 7.5 mg of prostaglandin analogue along with 44 IU of eCG at the time of implant removal. Khireddine et al. (1998) reported that administration of eCG at the time of removal of implant did not significantly improve the estrus or pregnancy rates.

7.1.3. Administration of GnRH in progestogen and estrogen regime

Troxel et al. (1993) reported that administration of 250 mcg of GnRH 30 h after the removal of norgestomet implant increased the pregnancy rates (46 %) following timed insemination in presynchronized anestrous and cyclic cows when compared with those of cows without GnRH injection (18 %). Similar increased conception rates following the administration of GnRH after norgestomet treatment were also been reported in cattle (doValle et al., 1997; Thompson et al., 1999).

7.1.4. Administration of estrogen following removal of progesterone treatment

Even though, most of the studies utilized estrogen at the beginning of progesterone treatment, reports regarding the use of estrogen at or after the withdrawal of progesterone treatment in cattle are also available (McDougall, 2001; McDougall et al., 2001).

Several authors reported that administration of estradiol benzoate 24 to 72 h following the withdrawal of progesterone treatment (9 to 14 d), increases the expression of estrus and enhanced incidence of ovulation without decreasing the pregnancy rate in postpartum cows by hastening or amplifying the preovulatory LH surge (Ulberg and Lindley, 1960; Saiduddin et al., 1968; Brown et al., 1972). Administration of estradiol 24 to 30 h after progesterone treatment for 7 d not only increased the number of animals exhibiting estrus in cows (Fike et al., 1997) and heifers (Johnson et al., 1997) but also improved the estrous synchrony (Peters et al., 1977; Macmillan and Burke, 1996). Subsequent work by Lammoglia et al. (1998) with heifers and cows treated with a 7 d progesterone treatment with prostaglandin on Day 6 of treatment followed by an injection of estradiol benzoate 24 to 30 h after removal of progesterone delivery device resulted in a greater synchronization rate when compared to those animals without estradiol benzoate injection. They experimented with varying dose levels of estradiol benzoate and concluded that the optimal responses were at 0.38 mg and 1 mg of estradiol benzoate for heifers and cows respectively. However, Lemaster et al. (1999) recommended 0.5 mg of estradiol benzoate 24 h after removal of progesterone treatment for effective coordinating ovulation with estrus in heifers.

Day et al. (2000) administered two doses of estradiol benzoate, with first injection given at the onset of 9 d intravaginal progesterone treatment, to manipulate follicular development, and the second injection given 48 h after the removal of the progesterone device and found that the strategic use of two injections of estradiol benzoate resulted in an increased precision of synchronized estrus with a conception rate comparable to those of a spontaneous estrus in cyclic and anestrous dairy cows.

8. Progestogen and prostaglandin combinations in dairy cattle

8.1. Reproductive performance following progesterone and prostaglandin treatment

Heersche et al. (1974) and Wishart (1974) for the first time attempted prostaglandin administration at or near the end of a progesterone treatment instead of administration of estrogen at the beginning of a progesterone treatment. They reported that cows treated with progesterone followed by prostaglandin for synchronization of estrus would come to estrum soon after the removal of the progesterone treatment because the treated animals could either have a CL that is susceptible to regression by prostaglandin or would have already undergone natural CL regression. Lane et al. (2001) recommended prostaglandin administration when short duration progesterone treatments were started in the early or mid cycle, as the proportion of animals requiring exogenous luteolysis induction increases during this period. Indeed, short term progesterone treatment using progesterone releasing intravaginal devices or subcutaneous ear implants combined with treatment with a luteolytic agent has proved successful in cattle (Roche, 1976a; Wishart, 1974; Thimonier et al., 1975; Beal, 1983).

Pregnancy rates equal to or greater than control rates for cows in natural estrus were achieved when progesterone releasing devices were used in conjunction with prostaglandin F2α or one of its analogues (Roche, 1976a; Wishart, 1974; Thimonier et al., 1975; Gyawu and Pope, 1983; Xu et al., 1996; Abdullah et al., 2001; Johnson and Spitzer, 2001). Several reports claim an improved response to estrus synchronization treatment when prostaglandin is administered 48 h after intravaginal progesterone device removal in Bos taurus (Gyawu et al., 1991; Tregaskes et al., 1994; Penny et al., 1997) and Bos indicus cattle (Kerr et al., 1991; Fitzpatrick and Finlay, 1993). Using progesterone releasing intravaginal device (PRID)-prostaglandin procedure, the conception rate was reported to be higher when PRID was inserted in the early (Day 1 to 10) rather than late (Day 11 to 20) stage of the estrous cycle (Folman et al., 1984).

When comparing the efficiency of prostaglandin treatment alone with that of combined progestin–prostaglandin treatment aimed at controlling estrous cycles in dairy cows, Chupin et al. (1977) found that combined treatment was more effective in bringing more cows into estrus during the first 96 h after the end of treatment than prostaglandin alone. Similarly, Gyawu et al. (1991) observed that the progesterone/prostaglandin combination was more effective in synchronizing ovulation compared to prostaglandin alone. Several authors have also reported increased synchronization rates and fertility following progesterone plus prostaglandin treatment (Smith et al., 1984; Munro and Moore, 1985; Ryan et al., 1999). However, Roche (1976a) observed a lower pregnancy rates even in effectively synchronized estrus in heifers treated with PRID for 7 d followed by a single injection of a prostaglandin analogue. Finally, Mialot et al. (1998) noted increased reproductive efficiency in cattle when prostaglandin instead of eCG was given 48 h after PRID removal.

8.2. Administration of estrogen or GnRH at the time of initiation of Progesterone - Prostaglandin schedule

Reduced fertility following short time progesterone treatment, conjunction with prostaglandin beginning later than Day 13 of estrous cycle (Beal et al., 1988) was reported due to the presence of persistent follicles (Rajamahendran and Taylor, 1991; Schmitt et al., 1994). To overcome this problem, GnRH (Macmillan and Thatcher, 1991; Twagiramungu et al., 1992) or estradiol-17β (Bo et al., 1994b) was administered at the beginning of the progesterone regime.

Xu and Burton (2000) suggested that the reproductive performance of cows receiving Ovsynch treatment could be improved by administration of progesterone treatment during the period between the GnRH and prostaglandin injections. They also suggested that the progesterone treatment can also prevent premature ovulation, after spontaneous luteolysis during the treatment period, in a small proportion of cows whose dominant follicles are not responsive to the GnRH treatment (Twagiramungu et al., 1992; Vasconcelos et al., 1997; Roy and Twagiramungu, 1999).

Based on the above assumptions, Xu and Burton (2000) conducted trails in lactating dairy cows with Ovsynch protocol interposition with progesterone treatment for 7 or 8 d during the synchronization period and inseminated cows at detected estrus. They recorded a tight synchrony of onset of estrus following synchronization of estrus with GnRH, 8 d progesterone, and prostaglandin but a reduced conception rate when compared with control without any treatment (56.5 versus 62.7 %) in lactating dairy cows. However, they recorded a reduced synchrony of onset of estrus with high conception rate (64.6 %) following synchronization of estrus with GnRH, 7 d progesterone, and prostaglandin treatment regime.

Ryan et al. (1995a) reported that administering GnRH was more effective than giving estradiol benzoate at the start of a progesterone-prostaglandin regime in dairy cows. In contrast, Lane et al. (2001) reported that 0.75 mg of estradiol benzoate administered at the start of 8 d of progesterone treatment, with prostaglandin given 1 d before progesterone withdrawal was more effective than GnRH for synchronizing estrus in heifers. Similarly, synchrony of estrus in dairy heifers sufficient for fixed time insemination was achieved using a protocol which involved the use of a progesterone controlled intravaginal drug releasing device (CIDR) for 10 d, a 10 mg estradiol benzoate capsule delivered at the time of device insertion, and prostaglandin administered 4 d before device removal (Macmillan and Peterson, 1993; Xu and Burton, 1999). In a study undertaken during the AI breeding period in lactating dairy cows, pregnancy rates were higher among cows synchronized with GnRH and a progesterone CIDR followed 7 d later by PGF2α treatment, and device removal 1 d after or at the time of prostaglandin treatment, compared to control unsynchronized cows (Xu and Burton, 2000).

9. Effect of progesterone, GnRH and prostaglandin in synchronization of postpartum dairy cows with ovarian disorders.

Several recent reports describe a high incidence of ovarian disorders in the preservice/postpartum period causing great economic impact in dairy farming due to extended intercalving period (Lamming and Darwash, 1998; Opsomer et al., 1998, 2000; López-Gatius et al., 2002; Wiltbank et al., 2002).

It has been recently possible to achieve estrus synchronization and acceptable pregnancy rates in dairy cows with different ovarian disorders detected during the early postpartum period, using various prostaglandin based protocols in combination with progesterone and GnRH.

Progesterone was included in a GnRH-prostaglandin-GnRH protocol for the treatment of abnormal ovarian conditions in postpartum dairy cows (Thatcher et al., 1993). Following the treatment regime: progesterone for 9 d, GnRH on D 0, and PGF2α on D 7, it was possible to successfully synchronize dairy cows with ovarian cysts during the postpartum period. Using the Ovsynch protocol as a therapeutic strategy for ovarian cysts, Bartolome et al. (2000) recorded similar pregnancy rates in response to timed insemination in cows with and without cysts. Further, López-Gatius and López-Béjar (2002) successfully synchronized and time inseminated lactating dairy cows with ovarian cysts using a protocol that combines GnRH and cloprostenol, starting treatment by simultaneously administering GnRH and cloprostenol. Pursley et al. (2001) observed that anovulatory cows fitted with an intravaginal progesterone device (CIDR) in the period between GnRH and PGF2α administration of the Ovsynch protocol showed higher pregnancy rates (55.2 %) than anovulatory cows subjected to Ovsynch without a CIDR (34.7%). In another study, López-Gatius et al. (2001) were also able to successfully synchronize and time inseminate lactating dairy cows with anovulatory follicles using a progesterone-GnRH-PGF2α treatment regime. Improved conception rates were reported in noncyclic dairy cows by administration of GnRH and a progesterone controlled intravaginal drug releasing insert (CIDR) on Day 0, CIDR removal and PGF2α treatment on Day 7, followed by estradiol benzoate on Day 9 for cows not showing signs of estrus by that time when compared to cows treated with CIDR and estradiol alone (Xu et al., 2000a, b)

III. APPROACH TO THE PROBLEM AND HYPOTHESIS

EXPERIMENT 1.

Following a voluntary waiting period, it becomes a necessity to breed the cows at the earliest possible time to reduce the intercalving period (Ferguson and Galligan, 1993). However, after resumption of ovarian activity, prolonged luteal phase is the primary factor that delays the first AI in postpartum dairy cows. Ovarian disorders and delayed uterine involution due to uterine disorders during the preservice period are attributed to this problem. The use of prostaglandin in hastening the uterine involution and its use as a therapeutic agent in certain uterine and ovarian disorders has been established in cattle (Eley et al., 1981; Lindel et al., 1982; Pankowshi et al., 1995).

Thus the first experiment titled "EFFECTS OF PRESYNCHRONIZATION DURING THE PRESERVICE PERIOD ON SUBSEQUENT OVARIAN ACTIVITY IN LACTATING DAIRY COWS" was designed to test the hypothesis that treatment with two doses of prostaglandin at 14 d apart during preservice period may enhance the number of cows in cyclicity and decrease certain uterine and ovarian disorders to some extent and consequently favor higher pregnancy rates.

EXPERIMENT 2.

Since presynchronization treatment during preservice period proved to be more beneficial for early postpartum dairy cows, all the experimental dairy cows used in the remaining research works were presynchronized during preservice period. Although results from first experiment show that presynchronization treatment solves the problem of prolonged luteal phase, uterine and certain ovarian disorders, its effect on anestrum due to persistent follicle is very much limited. Recently, López-Gatius et al., (2001) developed a timed insemination protocol, in which progesterone was administered for 9 d and GnRH was given on Day 0 and $PGF2\alpha$ on Day 7. This program was found to be more successful than the Ovsynch regimen for dairy cows with persistent follicles. Thus the second experiment titled "LUTEAL ACTIVITY AT THE ONSET OF A TIMED INSEMINATION PROTOCOL AFFECTS REPRODUCTIVE OUTCOME IN EARLY POSTPARTUM

DAIRY COWS" was designed to test the hypothesis that progesterone based estrus synchronization protocols may be better than Ovsynch protocol in early postpartum dairy cows. In addition, plasma progesterone concentrations were estimated in all cows, at the time of onset of the treatment, so as to study whether the luteal activity at the onset of treatment influences the reproductive outcome in early postpartum dairy cows.

EXPERIMENT 3.

Many papers concerning high evidence of ovarian disorders have been reported in early postpartum dairy cows (Lamming and Darwash, 1998; Opsomer et al., 1998, 2000). Anestrus due to anovulatory condition and cystic ovarian conditions namely follicular and luteal cysts are the most common ovarian disorders found during early postpartum period in dairy cows (Opsomer et al., 2000; Wiltbank et al., 2002). Of late specific timed insemination estrus synchronization protocols effective for the above said conditions are reported in early postpartum dairy cows (López-Gatius et al., 2001; López-Gatius and López-Béjar, 2002). These observations motivated to design the next research titled "SPECIFIC SYNCHRONIZATION OF ESTRUS ACCORDING TO OVARIAN STATUS IN EARLY POSTPARTUM DAIRY COWS" to test the theory that applying a specific estrous synchronization protocol depending on their ovarian status would be better than the mere implementation of Ovsynch protocol without the scrutiny of their ovarian status in early postpartum dairy cows.

IV. OBJECTIVES

This present work has been developed with a general objective to improve reproductive performance in early postpartum dairy cows by fixed timed insemination following different estrous synchronization protocols. Based on this primary aim, the investigation has been designed with the following specific objectives:

1. To evaluate the effects of presynchronization, with the administration of two doses of prostaglandin at a 14 d interval during the early postpartum period, on subsequent ovarian activity in clinically normal lactating dairy cows.

2. To determine whether interposition of progesterone treatment for 9 d by way of PRID between GnRH and PGF2α in GnRH-PGF2α-GnRH protocol (progesterone / GnRH / PGF2α protocol) would be more successful than the Ovsynch protocol for synchronization and timed insemination of presynchronized cows.

3. To investigate whether luteal activity, at the onset of timed insemination protocols, influence the reproductive outcome in early postpartum dairy cows.

4. To study the reproductive performance in presynchronized postpartum dairy cows subjected to a specific estrous synchronization protocol depending on their ovarian status and to compare with that cows, undergoing Ovsynch protocol without checking their ovarian status.

V. EFFECTS OF PRESYNCHRONIZATION DURING THE PRESERVICE PERIOD ON SUBSEQUENT OVARIAN ACTIVITY IN LACTATING DAIRY COWS

ABSTRACT

Among the strategies aimed at overcoming difficulties in estrus detection in dairy herds, presynchronization with 2 PGF2α treatments 14 d apart before a timed AI protocol has been related to a significant increase in pregnancy rates. The aim of the present study was to evaluate the effects of presynchronization during the preservice period on subsequent ovarian activity in clinically normal lactating dairy cows. A second objective was to evaluate the incidence of reproductive disorders on Day 50 postpartum. Depending on the chronological order of parturition, cows were alternately assigned to a control (n=102) or treatment (n=101) group. Animals in the treatment group were administered 2 cloprostenol treatments 14 d apart, beginning on Day 22 postpartum. The reproductive tract of each animal was examined ultrasonographically on Day 43 and 50 postpartum to monitor ovarian structures and uterine contents. Blood samples were collected on Day 50 for progesterone determination. Cows were inspected for signs of estrus between Days 50 and 71 postpartum and were then inseminated. Follicular persistence rates were similar in the presynchronized (14.9 %) and control (13.7 %) groups. Cows in the presynchronized group showed a lower metritis-pyometra rate (0 % < 3.9 %; P = 0.045); a lower ovarian cyst rate (3 % < 10.8 %; P = 0.03); a higher luteal activity rate (progesterone > 1 ng/mL) on Day 50 postpartum (76.2 % > 52.9 %; P = 0.0005); a higher estrus detection rate (73.3 % > 47.1 %; P < 0.0001); a higher ovulation rate (72 % > 44 %; P < 0.0001) and a higher pregnancy rate (29.7 % > 15.7 %; P = 0.02) than controls. Our results indicate that presynchronization during the preservice period reduces the incidence of ovarian cysts and metritis-pyometra determined on Day 50, and improves ovarian activity from Days 50 to 71 postpartum along with pregnancy rates in clinically normal lactating dairy cows.

Key words: presynchronization, prostaglandins, ovarian activity, dairy cattle

INTRODUCTION

Over the last 5 decades, average milk production has increased, but so has the incidence of reproductive disorders and infertility of dairy cows in the USA (Foote, 1996; Butler, 2000). The indicators conception and pregnancy rate to first service have suffered a decrease of 0.45 % (Bean and Butler, 1999) and 1 % (Royal et al., 2000) per year, respectively, and a high incidence of several ovarian disorders in the preservice/postpartum period has been recently reported (Opsomer et al., 1998, 2000). Regular cyclicity before 50 d postpartum is observed in only 51% of high yielding dairy cows; the risk factors calving season, problem calvings, clinical disease, ketosis or severe negative energy balance during the postpartum period are related to delayed cyclicity before service (Opsomer et al., 2000).

Prostaglandin F2α is a natural hormone that acts by promoting uterine involution postpartum (Eley et al., 1981; Lindel et al., 1982). There appears to be a relationship between the time to completion of uterine involution and the occurrence of the first postpartum ovulation with a normal luteal phase (Madej et al., 1984). In an attempt to hasten uterine involution and thus shorten the interval from parturition to estrus, exogenous PGF2α or its synthetic analogs have been administered early postpartum (usually as a single dose im) in both dairy and beef cows with variable results. Burton and Lean (1995) conducted a meta-analysis to establish the effect of PGF2α administered postpartum on the reproductive performance of dairy cattle. Although the weighted average reduction in days open between treated and control cows was 2.6 d for trials performed on cows showing an abnormal puerperium, and 3.3 d for trials including normal and abnormal postparturient cows, the results were not robust. The authors recommended that cows should not be treated with PGF2α before 40 d after calving until clinical trials provide further support for this practice.

Accurate estrus detection remains a major obstacle for improving fertility in many dairy farms, and timed AI protocols have been developed to overcome this problem. Moreira et al. (2001) reported a significant increase in pregnancy rates after presynchronization with 2 PGF2α treatments given 14 d apart before a timed AI protocol. The aim of the present study was to evaluate the effects of presynchronization during the preservice period on subsequent ovarian activity in clinically normal lactating dairy cows. A second objective was to evaluate the incidence of reproductive disorders on Day 50 post partum.

MATERIALS AND METHODS

Animals

This study was performed on a commercial dairy herd of 340 mature cows in northeastern Spain from October 1, 2000 to September 30, 2001. The voluntary waiting period from calving to first AI established for this dairy herd was 50 d. We performed the experiment using cows in their first or second lactation period. The cows were milked 3 times daily and kept in open stalls. We rejected cows with an abnormal puerperium. Excluding puerperal disorders were: twinning, retained placenta (fetal membranes retained longer than 12 h after parturition), primary metritis (diagnosed during the first or second week postpartum), or ketonuria (diagnosed during the second week postpartum). We confirmed the absence of these disorders with a planned monitoring program conducted during the 14 d following parturition. Cows with clinical conditions detected during the course of the study, such as mastitis, lameness, and digestive disorders, were also withdrawn from the program. The study population was finally formed by 203 cows. Mean daily milk production 10 d before Day 50 in milk was 46 kg.

Treatment

Depending on the chronological order of parturition, cows were alternately assigned to a control (n=102) or treatment (n=101) group. Animals in the treatment group were administered 2 cloprostenol (500 µg im; Estrumate, Schering Plough Animal Health, Madrid, Spain) treatments 14 d apart, beginning on Day 22 postpartum. Blood samples were taken from treatment and control cows on Day 50 and cows were examined ultrasonographically on Day 43 and 50.

Table 1. Definition of dependent variables.

Variable	Definition
Follicular persistence rate	Number of cows with a persistent follicle on Day 50 postpartum as a percentage of the total number of cows in each group.
Ovarian inactivity rate	Number of cows with inactive ovaries on Day 50 postpartum as a percentage of the total number of cows in each group.
Ovarian cystic condition rate	Number of cows with ovarian cysts on Day 50 postpartum as a percentage of the total number of cows in each group.
Pyometra rate	Number of cows with pyometra on Day 50 postpartum as a percentage of the total number of cows in each group.
Luteal activity rate	Number of cows with plasma progesterone concentrations ≥1 ng/mL on Day 50 postpartum as a percentage of the total number of cows in each group.
Cows in estrus	Number of cows showing estrus 50 to 71 d postpartum as a percentage of the total number of cows in each group.
Ovulation rate	Number of cows with at least 1 corpus luteum on Day 10 after AI as a percentage of the total number of cows in each group.
Pregnancy rate	Number of pregnant cows after AI as a percentage of the total number of cows in each group.

Ultrasound

The reproductive tract of each animal was examined by ultrasound on Days 43 and 50 postpartum to monitor ovarian structures and uterine contents. Using a portable B-mode ultrasound scanner (Scanner 100 Vet, equipped with a 5.0 MHz transducer; Pie Medical;

Maastricht, The Netherlands), each ovary was scanned in several planes by moving the transducer along its surface to identify the different structures. The size of follicular structures larger than 8 mm was measured using the built in electronic caliper after freezing the image on screen. The largest and the smallest diameters of the follicular antrum were measured and the mean diameter was then recorded.

The ovaries of inseminated cows were also examined by ultrasound 10 d after AI. All examinations were performed by the same operator.

A cow was considered to have a persistent follicle when a follicular structure of 8 to 15 mm was detected in the ultrasound examinations undertaken 7 d apart, in the absence of a corpus luteum or cyst, and no estrous signs between ultrasounds (López-Gatius et al., 2001). Ovaries were defined as inactive in the absence of behavioral signs of estrus, and of persistent follicles, corpora lutea or cysts in both ultrasound examinations. The ovarian cystic condition was diagnosed when a follicular structure of antrum diameter larger than 25 mm was detected in both preservice ultrasound examinations in the absence of a corpus luteum. A corpus luteum with or without a cavity was identified by its size and shape as well as by a granular, gray, structured area in the ovarian tissue (Kähn, 1994). Pyometra was diagnosed on Day 50 postpartum if fluid accumulation containing floccular echoes was observed in the uterine horns.

Progesterone Analysis

Blood samples were taken on Day 50 postpartum. All blood was collected into heparinized vacuum tubes from the coccygeal vein. Plasma was separated by centrifugation within 2 h and stored at –20 ºC until assayed. Progesterone was determined using solid-phase RIA kits containing antibody-coated tubes, ^{125}I-labeled progesterone and rabbit antiserum (CS Bio International, Gif-Yvette, France). The RIA method has been previously validated for use in the cow as described by Guilbault et al. (Guilbault et al., 1988). The sensitivity of the assay was 0.05 ng/mL progesterone. Plasma samples showing hormone concentrations below this value were assigned the sensitivity value. The intra-assay coefficient of variation was 16 %.

Plasma progesterone concentrations were used to classify the cows as showing (≥ 1ng/mL) or not showing (< 1ng/mL) luteal activity.

Detection of Estrus, AI and Pregnancy Diagnosis

The animals were inspected for signs of estrus (standing to be mounted) at least 4 times a day between Days 50 and 71 postpartum. Cows were inseminated by the same practitioner using frozen semen from a single ejaculate approximately 8 to 10 h after the first signs of estrus were observed. Pregnancy diagnosis was performed by palpation per rectum at 34 to 40 d postinsemination.

Data Analysis

The effect of treatment was evaluated in terms of the dependent variables defined in Table 1. The independent variables were the experimental groups. Treatment regimes were compared using the Chi-square test. Values are expressed as the mean ± standard deviation (SD).

RESULTS

Table 2 shows the effects of presynchronization during the preservice period on reproductive disorders and ovarian activity between Days 50 and 71 postpartum. Follicular persistence was similar in the treatment and control groups. No inactive ovaries were detected in any cow. Presynchronization treatment reduced the incidence of ovarian cysts and pyometra determined on Day 50, and significantly increased luteal activity, estrus, ovulation and pregnancy rates from Days 50 to 71 postpartum. No cows considered to have persistent follicles or pyometra showed estrous signs from Day 50 to 71 postpartum.

In cows showing no luteal activity, the average plasma progesterone concentration was 0.09 ± 0.02 ng/mL and ranged from 0.05 to 0.6 ng/mL. In cows with luteal activity, this variable was 3.2 ± 0.4 ng/mL, ranging from 1.3 to 4.8 ng/mL. Cows diagnosed as having persistent follicles showed no luteal activity. In the presynchronized and control groups, luteal activity was observed in 1 of 3, and 4 of 11 cows with ovarian cysts, respectively.

The mean number of days from parturition to first service was 57.9 ± 3.3 d for the treatment group and 60.8 ± 6.4 for the control animals.

Table 2. Effects of presynchronization (2 cloprostenol doses given 14 d apart) during the preservice period on reproductive disorders and luteal activity between Days 50 and 71 postpartum.

Group[a]	Control (n=102)	Treatment (n=101)	P
Persistent follicles (%)	13.7	14.9	0.9
Ovarian cysts (%)	10.8	3	0.03
Pyometra (%)	3.9	0	0.045
Luteal activity (%)	52.9	76.2	0.0005
Cows showing estrus (%)	47.1	73.3	< 0.0001
Ovulation rate (%)	44	72	< 0.0001
Pregnancy rate (%)	15.7	29.7	0.02

[a]Percentages are referred to the total number of cows in each group.

DISCUSSION

Presynchronization during the preservice period using 2 prostaglandin doses given 14 d apart, clearly improved ovarian activity from Days 50 to 71 postpartum. More cows subjected to presynchronization entered into estrus, ovulated and became pregnant, compared to untreated controls. In this study, we made a particular effort to minimize the effects of puerperal clinical conditions on subsequent ovarian activity. Cows developing mastitis, lameness and digestive disorders during the course of the study were also withdrawn. We can therefore state that our results closely reflect the direct effects of prostaglandins on ovarian activity, with the possible interference of subclinical disorders only. Once the ovary resumes activity in the early postpartum period, the most common ovarian disturbance appears to be a prolonged luteal phase before service (Opsomer et al., 1998, 2000; Smith and Wallace, 1998). This could be caused by a subclinical uterine infection provoking delayed luteolysis. The estradiol induction of oxytocin receptors through the luteolytic cascade required for prostaglandin F2α release and luteolysis can be adversely affected by abnormal uterine function (Roche et al., 2000). The administration of 2 prostaglandin doses 14 d apart in the early postpartum period has proved to be effective for the treatment of endometritis (Heuwieser et al., 2000). The same protocol used here for presynchronization allowed the application of the second prostaglandin dose on Day 36. This timing probably favors both the luteolysis of a possible persistent corpus luteum, and also gives rise to a clean uterine environment. Although our population was too small to draw any strong conclusions on the effect of presynchronization on reproductive disorders, the incidence of pyometra was significantly higher in the control group. The incidence of the ovarian cystic condition was also significantly lower in presynchronized cows, probably due to the luteolytic effect of prostaglandins on luteal cysts. These results nonetheless suggest that the presynchronization protocol favors a return to normal cyclicity at least in part through a postpartum therapeutic effect.

A lactating dairy cow may be diagnosed as having inactive ovaries when there are no behavioral signs of estrus, accompanied by failure to detect a corpus luteum or cyst on 2 consecutive examinations per rectum performed at an interval of 7 d (Markusfeld, 1987). In agreement with our previous results (López-Gatius et al., 2001), no cases of inactive ovaries were detected on Day 50 postpartum. The presence of a follicular structure similar in size to a dominant follicle (> 8 mm) was a characteristic feature of follicular persistence in all cows failing to show estrous signs for 7 d, in the absence of a corpus luteum or cyst. Persistent follicles are smaller than typical ovarian cysts and can be considered a feature of anestrus, which is due to the endocrine status of the cow (López-Gatius et al., 2001; Nobel et al., 2000). Presynchronization had no effect on persistent follicles, with similar proportions being noted in treated and control cows. In a therapeutic approach (López-Gatius et al., 2001), we also observed a limited response to GnRH plus PGF2α treatment in cows with persistent follicles. Progesterone appears to be the most efficient treatment for this ovarian disorder (López-Gatius et al., 2001; Anderson and Day, 1994; McDowell et al., 1998).

As expected, presynchronization improved the synchrony of the cycle at the end of the voluntary waiting period in the herd. The rate of luteal activity on Day 50 postpartum was significantly higher in presynchronized cows than in controls. This suggests that a higher number of cows would respond to estrus synchronization programs if presynchronized. However, the incidence of persistent follicles clearly affected the presynchronization response. Abnormal ovarian activity (persistent follicles plus ovarian cysts) remained high, close to 20 % in both groups. A reproductive examination at the beginning of the service period should improve subsequent timed AI protocols after excluding cows with ovarian disorders.

Although first postpartum ovulation usually occurs 15 to 25 d postpartum (Savio et al., 1990), regular ovarian cyclicity can be seriously altered during the second month of lactation depending on several factors such as herd, management, clinical diseases and milk production (Opsomer et al., 2000). This might explain some of the discrepancies in the literature concerning the effects of prostaglandin treatment in the early postpartum period on subsequent reproductive performance. In most studies, a single prostaglandin dose was administered earlier than 30 d after parturition (Burton and Lean, 1995). It is likely that a second dose given 14 d later would improve results. Here, we explored ovarian activity in clinically normal cows and found that the pregnancy rate in treated cows was double that recorded in control cows. Presynchronization appears to be cost effective. Our findings are consistent with previous results related to the use of 2 prostaglandin doses during the preservice period, either as presynchronization before a timed AI protocol (Moreira et al., 2001), or as a postpartum reproductive management tool for lactating dairy cows (Pankowski et al., 1995). Further research efforts need to be directed towards improving dairy cattle management practices to reduce the incidence of persistent follicles or anestrus at the start of the service period.

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VI. LUTEAL ACTIVITY AT THE ONSET OF A TIMED INSEMINATION PROTOCOL AFFECTS REPRODUCTIVE OUTCOME IN EARLY POSTPARTUM DAIRY COWS

ABSTRACT

This study was designed to compare two timed insemination protocols, in which progesterone, GnRH and PGF2α were combined, with the Ovsynch protocol in presynchronized, early postpartum dairy cows. Reproductive performance was also evaluated according to whether cows showed high or low plasma progesterone concentration, at the onset of treatment. One hundred and six early postpartum dairy cows were presynchronized with 2 cloprostenol treatments given 14 d apart, and then assigned to one of the three treatment groups. Treatments for the synchronization of estrus in all three groups were started 7 d after the second cloprostenol injection, which was considered Day 0 of the actual treatment regime. Cows in the control group (Ovsynch, n=30) were treated with GnRH on Day 0, PGF2α on Day 7, and were given a second dose of GnRH 32 h later. Cows in group PRID (n=45) were fitted with a progesterone releasing intravaginal device (PRID) for 9 d, and were given GnRH at the time of PRID insertion and PGF2α on Day 7. In group PRID/GnRH (n=31), cows received the same treatment as in the PRID group, but were given an additional GnRH injection 36 h after PRID removal. Cows were inseminated 16 to 20 h after the administration of the second GnRH dose in the Ovsynch group, and 56 h after PRID removal in the PRID and PRID/GnRH groups. Ovulation rate was determined on Day 11 postinsemination by detecting the presence of a corpus luteum in the ovaries. Lactation number, milk production, body condition at the onset of treatment and treatment regime were included as potential factors influencing ovulation and pregnancy after synchronization. Logistic regression analysis for cows with high and low progesterone concentration on treatment day 0 revealed that none of the factors included in the models, except the interaction between progesterone and treatment regime, influenced the risk of ovulation and pregnancy significantly. In cows with high progesterone concentration at treatment onset, Ovsynch treatment resulted in a significantly improved pregnancy rate over values obtained following PRID or PRID/GnRH treatment. In cows with low progesterone concentration, PRID or PRID/GnRH treatment led to markedly increased ovulation and pregnancy rates with respect to Ovsynch treatment. These findings suggest the importance of establishing ovarian status in early postpartum dairy cows before starting a timed AI protocol, in terms of luteal activity assessed by blood progesterone.

Key words: luteal activity, progesterone, radioimmunoassay, estrous synchronization, timed insemination, dairy cows

INTRODUCTION

Reproductive efficiency is essential for profitable dairy farming. Under most management systems, a 12 to 13-month calving interval is considered economically optimal (Olds et al., 1979; Holmann et al., 1984), though the reproductive performance of postpartum dairy cows is often limited by factors such as failure to ovulate or display estrus, along with poor estrus detection. Thus, many treatment protocols have been proposed to speed up the return to normal ovarian cyclicity after parturition and to synchronize ovulation for timed insemination in dairy cattle (Larson and Ball, 1992; Nebel and Jobst, 1998).

A timed artificial insemination protocol (Ovsynch) based on the use of GnRH and prostaglandins to synchronize ovulation was developed for use in dairy cows (Pursley et al., 1995). This program, extensively used at farm level (Nebel and Jobst, 1998), includes a GnRH treatment given at a random stage in the estrous cycle followed 7 d later by an injection of PGF2α. Thirty to thirty six hours later, a second dose of GnRH is administered and cows are inseminated 16 to 20 h after this last injection without detection of estrus. However, the success of the Ovsynch program was subsequently found to depend upon the stage of the estrous cycle at which the first GnRH dose is administered (Vasconcelos et al., 1999; Moreira et al., 2000). Reduced fertility occurs in dairy cattle when the Ovsynch protocol is started during the follicular and late luteal phases of the estrous cycle (Moreira et al., 2000) and pregnancy rates are enhanced when cyclic cows are presynchronized with two doses of prostaglandins given 14 days apart and started on the Ovsynch program at the early dioestrus stage (Moreira et al., 2001).

During the early postpartum period, anestrus attributable to an anovulatory condition is one of the most frequent disorders detected in lactating dairy cows (Wiltbank et al., 2002). In a previous study (López-Gatius et al., 2001), we developed a timed insemination protocol, in which progesterone was administered for 9 d and GnRH was given on Day 0 and PGF2α on Day 7. This program was found to be more successful than the Ovsynch regimen for dairy cows showing no luteal activity due to the presence of anovulatory follicles.

Since only 51% of dairy cows show regular ovarian cyclicity on Day 50 postpartum (Opsomer et al., 2000), we hypothesized that this timed insemination protocol could be a better alternative to the Ovsynch protocol for early postpartum cows. Thus our first objective was to determine whether the progesterone/GnRH/PGF2α protocol would be more successful than the Ovsynch protocol for synchronization and timed insemination of presynchronized cows. We also determined luteal activity at the beginning of treatment as a measure of reproductive performance. An additional objective was to explore the effect of a second dose of GnRH following progesterone withdrawal in the progesterone/GnRH/PGF2α protocol.

MATERIALS AND METHODS

Animals

This study was performed on a single, well-managed dairy herd in northeast Spain. The study population was formed by 106 lactating Friesian cows in their first or second lactation period, they calved between October 6, 2001, and April 12, 2002. The cows were kept in open stalls and milked 3 times daily. All cows were under strict daily veterinary supervision. The herd was maintained on a weekly reproductive health program. Cows undergoing an abnormal puerperium, such as assisted delivery, twinning, retained placenta (fetal membranes retained longer than 12 h after parturition), primary metritis (diagnosed during the first or second week postpartum), or ketonuria (diagnosed during the second week postpartum) were excluded. Cows with clinical conditions detected during the course of the study, such as mastitis, lameness, digestive disorders, abnormal genital discharges and pathological abnormalities of the reproductive tract detectable on palpation per rectum, were also withdrawn from the program. All animals were in excellent health and body condition at the time of synchronization of estrus and insemination. The cows were scored for body condition on a five-point scale: 1=thin to 5=fat (Edmonson et al., 1989). Cows awarded scores of 2.5 to 3.5 were considered to be in suitable condition. Body condition scores were assigned by the same technician.

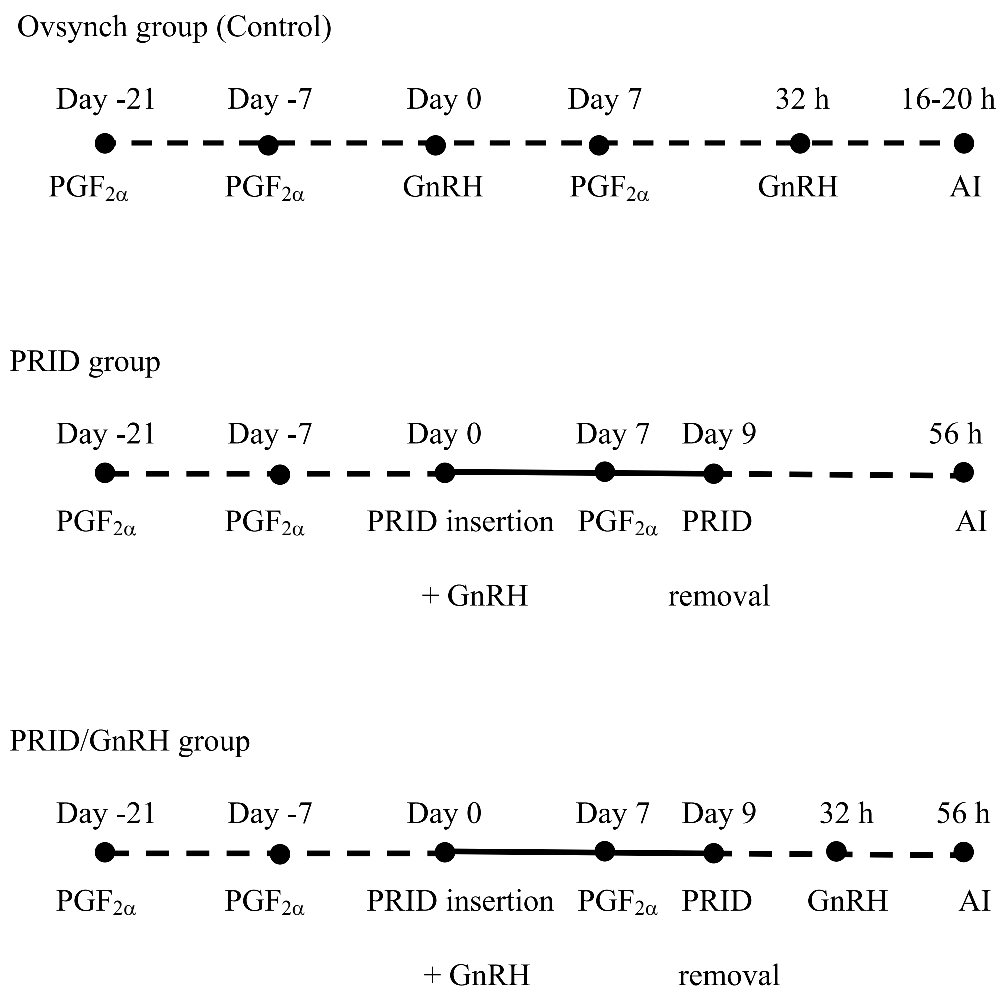
Treatments

All the animals were presynchronized with 2 cloprostenol (500 µg; Estrumate, Schering Plough Animal Health, Madrid, Spain) im treatments given 14 d apart, starting from Day 18 to 22 postpartum. Cows were then alternately assigned to 1 of 3 treatment groups on a weekly rotational basis according to the chronological order of their calving data (Fig. 1). Treatments for the synchronization of estrus in all three groups were started 7 days after the second cloprostenol treatment of the presynchronization protocol. This day was taken as Day 0 of the treatment regime. Cows in the control group (Ovsynch, n=30) were treated with GnRH (100 µg im; Cystorelyn, Sanofi Salud Animal, Barcelona, Spain) on Day 0 followed by a luteolytic dose of PGF2α (25 mg im; Enzaprost, Sanofi Salud Animal, Barcelona, Spain) on Day 7 and a second dose of GnRH im 32 h later. Cows in group PRID (n=45) were fitted with a progesterone releasing intravaginal device (PRID, containing 1.55 g of progesterone, Sanofi Salud Animal, Barcelona, Spain) on Day 0. The PRID was maintained for 9 days without the estradiol benzoate capsule. These animals were also given 100 µg GnRH im at the time of PRID insertion, and 25 mg PGF2α im on Day 7. Cows in PRID/GnRH (n=31) received the same treatment as the PRID group but were administered an additional GnRH dose 36 h after PRID removal.

Artificial Insemination and Pregnancy Diagnosis

Cows were inseminated 16 to 20 h after administration of the second GnRH injection in the Ovsynch Group, and 56 h after PRID removal in the PRID and PRID/GnRH groups. Inseminations were performed between 46 and 53 days postpartum, the average being 50 days postpartum.

Figure 1. Schematic representation of the synchronization protocol used in the three treatment groups.



Ovulation was determined on Day 11 postinsemination by detecting the presence of a corpus luteum in the ovaries by ultrasound. Ovulation rate was defined as the number of cows with at least one corpus luteum as a percentage of the total number of cows in each group. If a cow had returned to estrus between Days 7 and 30 postinsemination, estrus was confirmed by examination. per rectum (López-Gatius and Camón-Urgel, 1991) and the animals were reinseminated with no additional treatment. Cows that exhibited estrus outside this interval and prior to pregnancy diagnosis were not inseminated. Pregnancy diagnosis was performed 34 to 40 d postinsemination by ultrasound. Pregnancy rate was defined as the number of pregnant cows after first AI as a percentage of the total number of cows in each group.

Blood Progesterone

Blood samples were obtained from all cows at the start of synchronization treatment (Day 0). Blood was collected from the coccygeal vein into heparinized vacuum tubes. Plasma was separated by centrifugation within 2 h and stored at –20ºC until assayed. Progesterone was determined using a solid-phase RIA kit containing antibody-coated tubes, ^{125}I-labelled progesterone and rabbit antiserum (CS Bio International, Gif-Yvette, France). The RIA method was previously validated for its use in the cow as described by Guilbault et al. (1988). The sensitivity of the assay was 0.05 ng/mL progesterone. Plasma samples showing hormone concentrations below this value were assigned the sensitivity value. The intraassay coefficient of variation was 16%. Plasma progesterone concentration was used to classify the cows as showing high (≥ 1ng/ml) or low (< 1ng/ml) progesterone concentration.

Data Collection and Analysis

Overall reproductive performance for the three treatment groups was evaluated using the Chi-square test. The effect of treatment group and level of progesterone on ovulation and pregnancy rate were analyzed by logistic regression (Proc Genmod, SAS, 1992) adjusting for lactation, days in milk, milk production and body condition score. The estimates and Wald 95% limits were used to calculate odd ratios and 95% CI. The explanatory variables and interaction were evaluated using the backward elimination procedure and variables that significantly affected pregnancy or ovulation rate remained in the model (Agresti, 1996). The level of significance was set at P<0.05. Values are expressed as the mean ± standard deviation (SD).

RESULTS

The mean lactation number was 1.7 ± 0.5 (x ± SD; ranges from 1 to 2 lactations). The mean body condition score at treatment onset was 3.2 ± 0.3 (ranges from 2.5 to 3.5). The mean milk production at treatment onset was 43.9 ± 10.6 kg (ranges from 20 to 69 kg). In cows with high progesterone concentration (n= 69), average plasma progesterone was 2.79 ± 0.67 ng/mL (1.70 to 4.24 ng/mL), 2.93 ± 0.59 ng /mL (1.80 to 4.22 ng/mL) and 2.83 ± 0.69 ng / mL (1.70 to 4.22 ng/mL) for the Ovsynch, PRID and PRID/GnRH groups, respectively. In cows with low progesterone concentration (n=37), corresponding progesterone levels were 0.16 ± 0.07 ng/mL (0.09 to 0.23 ng/mL), 0.17 ± 0.08 ng/mL (0.09 to 0.34 ng/mL), and 0.14 ± 0.07 ng/mL (0.09 to 0.23 ng/mL), respectively.

Table 1 shows overall reproductive performance for the three treatment groups. Ovulation rate showed a significant increase (P<0.01) in the PRID/GnRH group (83.97%), compared to the Ovsynch (60%) or PRID (64.4%) groups. However, similar pregnancy rates and proportions of animals returning to estrus were recorded in the three treatment groups.

Considering the presence of high or low progesterone, tables 2 and 3 summarize the ovulation and pregnancy rate, respectively, odd ratios and 95% confidence intervals. The final models for both included only the interaction between progesterone and treatment. Lactation, milk production, days in milk and body condition score were not significant and were not included in the final models. Ovsynch reduces ovulation rate in cows with low progesterone, increases pregnancy rate in cows with high progesterone, and PRID+GnRH and PRID increase pregnancy rate in cows with low progesterone.

There was a significant (P<0.01; Figures 2 and 3) interaction between treatment group and the level of progesterone on Day 0 for the probability of ovulation and pregnancy. The interaction implies that in cows with low progesterone Ovsynch may be reducing ovulation rate and the protocols including progesterone may be increasing pregnancy rate; whereas in cows with high progesterone Ovsynch increase pregnancy rate.

Table 1. Cows with high (>1 ng/ml) or low (<1 ng/ml) progesterone concentration [P4] at the time of treatment onset and effects of the three treatment regimes on rates of ovulation, pregnancy and return to estrus.

Group	Ovsynch(n=30)	PRID(n=45)	PRID/GnRH(n=31)
High [P4] (%)[a]	63.3	66.7	64.5
Ovulation rate(%)[b]	60 [j]	64.4 [j]	83.9 [k]
Pregnancy rate(%)[c]	26.7	24.4	35.5
Return to estrus(%)[d]	20	15.6	16.1

Group Ovsynch = 100 μg GnRH im on Day 0; 25 mg PGF2α im on Day 7 followed by 100 μg GnRH im 32 h later; AI 16 to 20 h after the second GnRH treatment.

Group PRID = 1.55 g intravaginal progesterone for 9 d, plus 100 μg GnRH im on Day 0, followed by 25 mg, im PGF2α on Day 7; AI 56 h after PRID removal.

Group PRID/GnRH = same as for the PRID group plus 100 μg GnRH im 36 h after removal of PRID.

[a,c,d]: No significant differences detected by the Chi-square test.

[b]: Different superscripts denote significant differences detected by the Chi-square test (j-k: $P<0.01$).

Table 2. Effects of treatment on ovulation rate in cows with high (>1 ng/ml) or low (<1 ng/ml) progesterone concentration [P4] at the onset of treatment.

Treatment	Progesterone	Ovulation rate		OR	95% CI	P value
		n	%			
PRID+GnRH	High	15/20	75	Referent	Referent	
PRID+GnRH[a]	Low	11/11	100	-	-	-
PRID	High	15/30	50	0,33	0,1-1,15	0,08
PRID	Low	14/15	93.3	4,7	0,5-45	0,18
Ovsynch	High	16/19	84.2	1,8	0,4-8,8	0,48
Ovsynch	Low	2/11	18.2	0,07	0,1-0,46	0,005

Group Ovsynch = 100 μg GnRH im on Day 0; 25 mg PGF2α im on Day 7 followed by 100 μg GnRH im 32 h later; AI 16 to 20 h after the second GnRH treatment.

Group PRID = 1.55 g intravaginal progesterone for 9 d, plus 100 μg GnRH im on Day 0, followed by 25 mg, im PGF2α on Day 7; AI 56 h after PRID removal.

Group PRID/GnRH = same as for the PRID group plus 100 μg GnRH im 36 h after removal of PRID.

[a]Un-reliable estimate

Table 3. Effects of treatment on pregnancy rate in cows with high (>1 ng/ml) or low (<1

ng/ml) progesterone concentration [P4] at the onset of treatment.

Treatment	Progesterone	Pregnancy rate n	%	OR	95% CI	P value
PRID+GnRH	High	2/20	10	Referent	Referent	
PRID+GnRH	Low	9/11	81.8	40.4	4.8-333.6	0.0006
PRID	High	3/30	10	1	0.1-6.5	1
PRID	Low	8/15	53.3	10.3	1.7-60.9	0,01
Ovsynch	High	8/19	42.1	6.5	1.2-36.6	0.01
Ovsynch[a]	Low	0/11	0	-	-	-

Group Ovsynch = 100 µg GnRH im on Day 0; 25 mg PGF2α im on Day 7 followed by 100 µg GnRH im 32 h later; AI 16 to 20 h after the second GnRH treatment.
Group PRID = 1.55 g intravaginal progesterone for 9 d, plus 100 µg GnRH im on Day 0, followed by 25 mg, im PGF2α on Day 7; AI 56 h after PRID removal.
Group PRID/GnRH = same as for the PRID group plus 100 µg GnRH im 36 h after removal of PRID.
[a]Un-reliable estimate

Figure 2. Interaction between treatment group and level of progesterone on Day 0 for the

probability of ovulation.

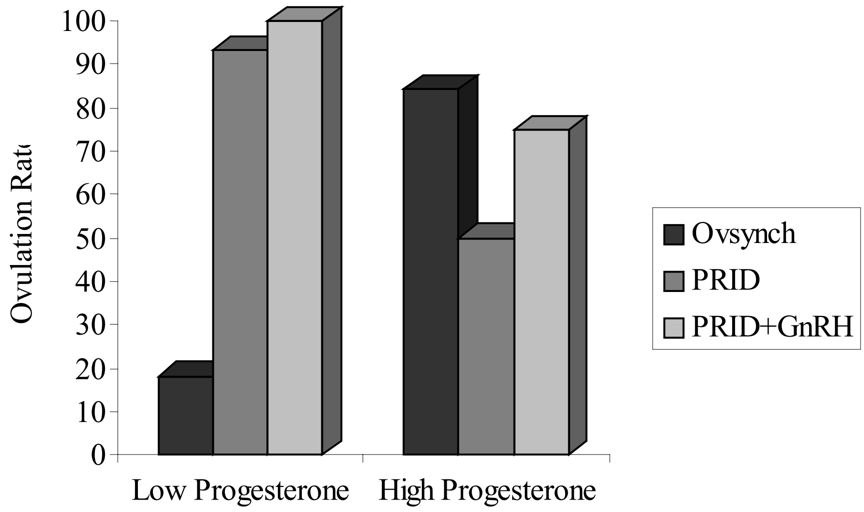
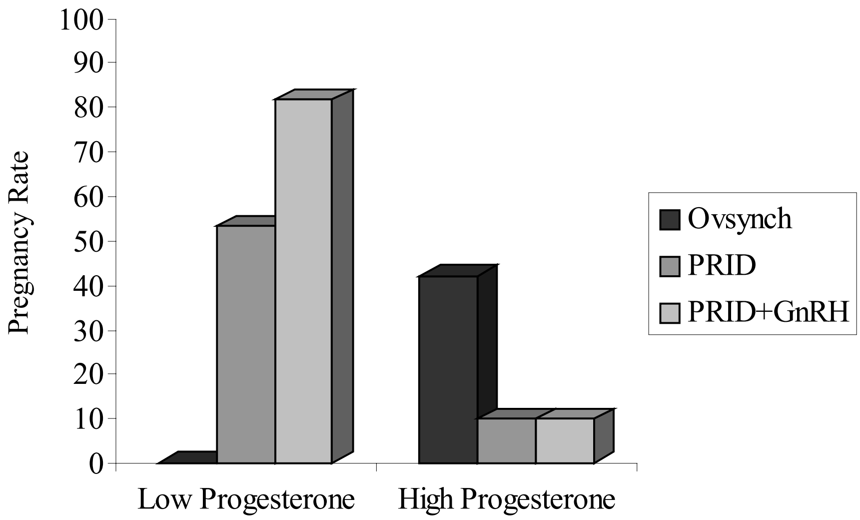


Figure 3. Interaction between treatment group and level of progesterone on Day 0 for the probability of pregnancy.



DISCUSSION

This report describes a preliminary evaluation of timed insemination protocols based on combining progesterone, GnRH and PGF2α for use in presynchronized early postpartum dairy cows. Efforts were made to reduce variation in the general status of the animals so that failure to ovulate or conceive could be attributed to factors other than the clinical condition of the cows during the study. However, as indicated by the un-reliable estimates for the PRID/GnRH-low progesterone group (100% ovulation rate) and Ovsynch-low progesterone group (0% pregnancy rate), the limited number of animals could probably affect some of the results. Nevertheless, the present results accomplish the main objective of the study: when the data were stratified according to whether the animals showed high or low progesterone concentration at treatment onset, a significant difference in ovulation and pregnancy rates was observed between the Ovsynch and progesterone treatment groups (PRID and PRID/GnRH). Ovsynch increases the pregnancy rate in cows with high progesterone (cycling), whereas progesterone based protocols are more successful than the Ovsynch for cows with low progesterone.

The administration of an initial dose of GnRH in the Ovsynch protocol promotes ovulation or luteinization of large follicles, synchronizes the recruitment of a new follicular wave, and controls the developmental stage of a preovulatory follicle before PGF2α causes the regression of an original or a GnRH induced corpus luteum (Macmillan and Thatcher, 1991). The second GnRH dose induces a preovulatory LH surge (Schmitt et al., 1996) and subsequent ovulation of a newly recruited follicle within 30 h of injection (Pursley et al., 1995). In the present study, treatment was started 7 d after the second dose of PGF2α used for presynchronization. Many of the cyclic cows with a corpus luteum that responded to PGF2α during the presynchronization period may have been in the early luteal phase of the estrous cycle on the initiation of treatment. The improved pregnancy rate after timed insemination recorded in the Ovsynch-high progesterone group confirm the results of previous studies in which sequential treatment with GnRH and PGF2α was shown to synchronize follicular development and regression of the corpus luteum in dairy cows

(Schmitt et al., 1996; Burke et al., 1996; Pursley et al., 1997a,b).

In contrast, treatment of cows showing high progesterone concentration with progesterone as well as GnRH in the PRID and PRID/GnRH groups gave rise to lower pregnancy rates. Our results are somewhat inconsistent with those of several studies (Ryan et al., 1995; Xu et al., 1997; Xu and Burton, 1998, 2000), in which satisfactory pregnancy rates were obtained following synchronization by progesterone plus PGF2α with or without the administration of estrogen or GnRH at the onset of progesterone treatment. However, in these reports, the cows had been inseminated at detected estrus rather than undergoing timed insemination. Nevertheless, the administration of estradiol benzoate at the onset of 10 d of progesterone treatment followed by PGF2α appears to be satisfactory for the timed insemination of dairy heifers (Xu and Burton, 1999). There is indeed scope for much further research directed towards understanding the mechanisms by which progesterone, GnRH and PGF2α protocols initiated at the early luteal phase of the estrous cycle affect follicular waves in early postpartum dairy cows, possibly leading to reduced pregnancy rates after timed insemination.

Different response to treatments was observed in cows with low progesterone concentration at the start of treatment. Higher ovulation and pregnancy rates were noted after the progesterone treatments compared to the Ovsynch protocol. However, since the current experimental design started timed AI protocols 7 d after the second PGF2α treatment, cyclic cows with low progesterone values must have been in the early metestrus of the estrus cycle at the time of GnRH, and would be less fertile to an Ovsynch program (Vasconcelos et al., 1999; Moreira et al., 2000, 2001). It has been suggested that the optimal stage of the estrus cycle at which the Ovsynch protocol should be initiated corresponds to the early luteal phase (i.e., between Day 5 and 10 of the estrus cycle) (Moreira et al., 2000). A further reason for the poor pregnancy rate following Ovsynch treatment in cows with low progesterone concentration could be anovulatory condition (López-Gatius et al., 2001; Wiltbank et al., 2002). Most anovulatory cows probably lack GnRH responsive follicles at the time of the first GnRH dose of the Ovsynch protocol. It has been shown that ovulation of the follicle in response to the first GnRH injection is a prerequisite for the success of the

Ovsynch program (Vasconcelos et al., 1999). Moreover, we also found a limited response to treatment with GnRH plus PGF2α in cows with a persistent follicle (López-Gatius et al., 2001).

In the PRID and PRID/GnRH treatment groups, ovulation and pregnancy rates were higher in cows with low progesterone concentration at the time of treatment onset. The combination of GnRH and progesterone in the PRID and PRID/GnRH regimes seems to be sufficient to facilitate follicular growth and maturation before ovulation after removing the progesterone device, leading to improved ovulation and pregnancy as reported by Stevenson et al. (1997) in anestrous beef cows. Our results show that timed insemination after 9 d of progesterone treatment plus GnRH on Day 0 and PGF2α on Day 7 is an effective way of managing reproduction in cows with luteal activity, either noncyclic or in the follicular phase of the estrous cycle, but warrants further investigation for large herd populations with more emphasis on follicular dynamics.

On overall analysis of the data, it was revealed that the ovulation rate in PRID/GnRH (83.9 %) was significantly higher ($P<0.001$) than that recorded for the PRID (64.4 %) or Ovsynch (60%) groups. On the contrary, pregnancy rates in PRID (24.4%), PRID/GnRH (35.5%) and Ovsynch (26.7%) showed no significant difference and were also comparable to rates recorded for lactating cows in natural estrus (36%) in the geographical region of our study (López-Gatius, 2000). It should be highlighted that administering GnRH at the time of the preovulatory LH surge led to an improved ovulation rate compared to PRID treatment, which lacked a second GnRH dose. Indeed, the second dose of GnRH improved ovulation of the dominant follicle. This may be attributed to an amplified spontaneous endogenous preovulatory LH surge by the second GnRH dose administered around the time of the endogenous LH surge (Lucy and Stevenson, 1986; Rosenberg et al., 1991). An additional possibility is that cows that do not necessarily have positive feed back to estradiol could ovulate to exogenous GnRH and that this may be more noticeable in cows with luteal activity. The possible beneficial effect of administering GnRH upon removal of the progesterone device requires further investigation on a larger study population.

The present presynchronization program involving a double PGF2α dose was designed to speed up involution of the postpartum uterus and reduce the effects of any subclinical uterine pathology (Paisley et al., 1986) that could interfere with results. By estimating plasma progesterone at the onset of treatment we were able to establish that about 65% cows showed high progesterone concentration in all three treatment groups. We would even expect an increase of this figure by sampling blood later than 7 d after the second PGF2α treatment. As noted above, cyclic cows could have been in early metestrus at treatment onset to give progesterone values lower than 1ng/mL. This relatively higher number of animals with high progesterone concentration during the early postpartum period compared to a previous report by Opsomer et al. (2000) may be explained by the role played by PGF2α in resuming ovarian activity in postpartum dairy cows (Paisley et al., 1986). The fact that only clinically normal cows were included in this study would also be expected to contribute to this discrepancy.

In conclusion, our findings suggest a need to determine the ovarian status of the early postpartum dairy cow before commencing a timed insemination protocol. This status can be appropriately established by assessing luteal activity by determining plasma progesterone.

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VII. SPECIFIC SYNCHRONIZATION OF ESTRUS ACCORDING TO OVARIAN STATUS IN EARLY POSTPARTUM DAIRY COWS

ABSTRACT

The problem of poor estrus detection in dairy herds has been frequently addressed by adopting programs that regulate the estrous cycle. The present study was designed to compare the reproductive performance of presynchronized postpartum dairy cows subjected, either to the Ovsynch protocol without screening for ovarian status, or to a specific estrous synchronization protocol applied according to their ovarian status, as determined by transrectal ultrasound. The study was conducted on 428 lactating dairy cows. All the animals were presynchronized with 2 cloprostenol im treatments given 14 d apart, starting from Day 14 to 20 postpartum. The cows were then assigned to 1 of 2 treatment groups. Cows in the Ovsynch group (n=205) received GnRH im, Day 0; cloprostenol im, Day 7; GnRH im, 36 h later; AI 16 to 20 h after the second GnRH. Cows in the specific synchronization (Ssynch) group (n=223) were weekly subjected to transrectal ultrasound exams for 4 weeks, or until AI or starting treatment, and divided into four subgroups according to their ovarian status: 1) CL subgroup (n=130), cows with a corpus luteum. These cows received 500 μg im cloprostenol and 250 IU hCG plus 1 mg EB im 12 h later, and were inseminated 48 h after cloprostenol treatment; 2) NE subgroup (n=58), cows inseminated at natural estrus; 3) PF subgroup (n=26), cows considered to suffer follicular persistence. This subgroup was treated with 1.55 g intravaginal progesterone (PRID) for 9 d; 100 μg GnRH im on Day 0; 500 μg cloprostenol im on Day 7; AI 56 h after PRID removal; and 4) OC subgroup (n=9), cows with ovarian cysts. These cows were given 100 μg GnRH plus 500 μg cloprostenol im on Day 0; 500 μg cloprostenol im on Day 14 followed by 100 μg GnRH im 36 h later; AI 24 h after the second GnRH dose. The Ovsynch and Ssynch regimes were started 11 and 14 d, respectively, after the second cloprostenol dose of the presynchronization protocol. Logistic regression analysis was carried out for the dependent variables ovulation and pregnancy rates to first and to second AI (second AI: first AI plus return AI). Treatment regime, lactation number, milk production and body condition on day 50 postpartum, and AI season were considered factors. There were no significant effects of treatment regime on ovulation rate, nor were there any effects of lactation number, milk production and body condition on pregnancy rates. Insemination season was a significant risk factor for pregnancy to first and to second AI. No interactions were found. Cows subjected to specific synchronization were 2.1 times more likely to become pregnant at first and second AI compared to those synchronized using the Ovsynch protocol ($P<0.0001$). Our results clearly show that the response of postpartum presynchronized cows to a specific estrous synchronization protocol applied according to their ovarian status is much better than their response to a single protocol applied without taking into account the ovarian status of the animals. The cost of a weekly based veterinary program could be recovered by monitoring ovarian status before applying a program for estrous synchronization plus timed AI.

Key words: dairy cows, early postpartum, specific synchronization of estrus, timed insemination

INTRODUCTION

In many dairy herds, poor estrus detection is the main reason for increasing the calving interval (Senger, 1994; Heersche and Nebel, 1994; Sturman et al., 2000). Programs aimed at regulating the estrous cycle attempt to correct this failure by allowing the accurate detection of cows in estrus (Nebel and Jobst, 1998). The recently developed ovulation synchronization protocol denoted Ovsynch (Pursley et al., 1995), is currently extensively applied for the timed insemination of lactating dairy cows (Nebel and Jobst, 1998). The Ovsynch method consists of GnRH treatment given at random stages of the estrous cycle followed by PGF2α 7 d later. A second dose of GnRH is administered 36 h after PGF2α treatment and the cows are inseminated 16 to 20 h later without detection of estrus. By presynchronizing early postpartum dairy cows with double prostaglandin treatments, given 14 d apart to initiate Ovsynch at the early luteal stage, the pregnancy rates of cyclic cows were improved over rates obtained for cows undergoing Ovsynch at random stages of the estrous cycle (Moreira et al., 2001).

Several recent reports describe a high incidence of ovarian disorders in the preservice/postpartum period (Lamming and Darwash, 1998; Opsomer et al., 1998, 2000). Regular cyclicity after 50 days postpartum was observed in only 51% of all high producing dairy cows examined (Opsomer et al., 2000). In a previous study (López-Gatius et al., 2001), we found that a timed insemination protocol, in which progesterone was administered for 9 Days, with GnRH given on day 0 and PGF2α on day 7, was more successful than Ovsynch in postpartum dairy cows that were anestrous because they had persistent follicles. We also observed (López-Gatius and López-Béjar, 2002) that postpartum dairy cows with ovarian cysts were successfully synchronized and time inseminated by simultaneous administration of GnRH and prostaglandin on Day 0, prostaglandin on Day 14 and GnRH 36 h later. In another study (López-Gatius, 2000), we demonstrated that the administration of prostaglandin followed by 250 IU hCG and 1 mg estradiol benzoate 12 h later, was effective in postpartum dairy cows in the luteal phase for the synchronization of estrus, followed by timed AI. Based on our past experience with estrous synchronization, we hypothesized that the specific synchronization of estrus according to the ovarian status of the animals would lead to enhanced improvement of reproductive performance, in relation to the use of simpler protocols.

We therefore designed the present study to compare the reproductive performance of presynchronized postpartum dairy cows subjected to the Ovsynch protocol without checking their ovarian status, with that of cows undergoing a specific estrous synchronization protocol depending on their ovarian status, as assessed by transrectal ultrasound.

MATERIALS AND METHODS

Animals

The study was conducted on 428 lactating Holstein-Friesian cows from a single, well-managed dairy herd over a 19-month period (1 July 2000 to 31 January 2002). The cows were kept in open stalls and milked 3 times daily. All cows were under strict daily veterinary supervision. Cows undergoing an abnormal puerperium, such as assisted delivery, twinning, retained placenta (fetal membranes retained longer than 12 h after parturition), primary metritis (diagnosed during the first or second week postpartum), or ketonuria (diagnosed during the second week postpartum) were excluded. Cows with clinical conditions detected during the course of the study, such as mastitis, lameness, digestive disorders, abnormal genital discharges and pathological abnormalities of the reproductive tract detectable on palpation per rectum, were also withdrawn from the program. All animals were in excellent health and body condition at the time of synchronization of estrus and insemination. The cows were scored for body condition using a five-point scale: 1=thin to 5=fat (Edmonson et al., 1989). Cows awarded scores of 2 to 3.5 were considered to be in suitable condition. Efforts were made to reduce variation in the general status of the animals, such that failure to ovulate or to conceive could be attributed to factors other than the clinical condition of the cows during the study.

Treatments and insemination

All the animals were presynchronized with 2 cloprostenol (500 µg; Estrumate, Schering Plough Animal Health, Madrid, Spain) im treatments given 14 d apart, starting from Day 14 to 20 postpartum. Cows were then alternately assigned to 1 of 2 treatment groups on a weekly rotational basis according to the chronological order of their calving data. Treatment for the synchronization of estrus in the Ovsynch group (Control, n=205) started 11 d after the second cloprostenol dose of the presynchronization protocol. On this day, taken as Day 0 of the treatment regime, cows received GnRH (100 µg im; Cystorelyn, Sanofi Salud Animal, Barcelona, Spain), followed by an im luteolytic dose of cloprostenol

(500 μg) on Day 7, and a second dose of GnRH 36 h later. Cows in this group were inseminated 16 to 20 h after the second GnRH dose.

Cows in the specific synchronization (Ssynch) group (n=223) were subjected to transrectal ultrasound exam 14 d after the second prostaglandin dose of the presynchronization protocol (this day was taken as Day 0 for this group). Animals were then ultrasound examined weekly for 4 weeks, or until AI or starting treatment, using a portable B-mode ultrasound scanner (Scanner 100 Vet equipped with a 5.0 MHz transducer; Pie Medical, Maastricht, The Netherlands). Each ovary was scanned in several planes by moving the transducer across its surface. All the ultrasound exams were performed by the same practitioner throughout the study. The cows were then divided into four subgroups according to their ovarian status:

1) CL subgroup (n=130): cows with a corpus luteum estimated to be at least 15 mm (mean of maximum and minimum diameters) on Day 0 or in subsequent ultrasound exams. A corpus luteum with or without a cavity was identified by its size and shape as well as by a granular, gray, structured area that could be identified in the ovarian tissue (Kähn, 1994). These cows received 500 μg im cloprostenol at corpus luteum diagnosis and 250 IU hCG plus 1 mg EB (Neonida N, Smith Kline Beecham Sanidad Animal, Madrid, Spain) im 12 h later. Insemination was 48 h after cloprostenol treatment (López-Gatius, 2000).

2) NE subgroup (n=58): cows showing natural estrus between Day 0 and 28. Estrus was confirmed by palpation per rectum (López-Gatius and Camón-Urgel, 1991) and the cows were inseminated at this time.

3) PF subgroup (n=26): cows with follicular persistence. A cow was considered to have a persistent follicle when a follicular structure from 8 to 15 mm, at least, was detected in 2 consecutive ultrasound exams in the absence of a corpus luteum or cyst, and no estrus signs were noted during the 7 d interval between ultrasound exams. These cows were fitted with a progesterone releasing intravaginal device (PRID, containing 1.55 g of progesterone, Sanofi Salud Animal, Barcelona, Spain) at diagnosis. The PRID was maintained for 9 days without the estradiol benzoate capsule. These animals were also given 100 μg GnRH im at

the time of PRID insertion, and 500 µg cloprostenol im 7 d later. These cows were inseminated 56 h after PRID removal (López-Gatius et al., 2001).

4) OC subgroup (n=9): cows with ovarian cysts. A cow was considered to have an ovarian cyst when a follicular structure estimated to be greater than 25 mm could be observed in 2 consecutive ultrasound exams in the absence of a corpus luteum, and no estrus signs were noted during the 7 d period between the exams. Cows received 500 µg cloprostenol and 100 µg GnRH im at cyst diagnosis, a second dose of cloprostenol 14 d later and GnRH 36 h after this. Insemination was undertaken 24 h after the second GnRH dose (López-Gatius and López-Béjar, 2002).

Ovulation rate was determined on Day 11 after the first AI by ultrasound detection of a corpus luteum in the ovaries. If a cow had returned to estrus between Days 8 and 30 postinsemination, estrus was confirmed by palpation per rectum and the animals were reinseminated with no additional treatment. Cows that exhibited estrus outside this interval and prior to pregnancy diagnosis were not inseminated. Pregnancy diagnosis was performed 34 to 40 d postinsemination by ultrasound. The insemination was done with semen from a single bull of proven fertility.

In the geographical area of our study there are only two clearly distinguishable meteorological seasons. In a previous study (Labérnia et al., 1998), we divided the year into warm (May to September) and cool (October to April) periods and observed that during the warm period, reproductive variables were significantly impaired. For this reason, we used the first insemination dates to analyze the effect of the season of AI on the occurrence of ovulation, return to estrus and pregnancy.

Data analysis

The effects of the treatment regime (Ovsynch versus Ssynch) were evaluated in terms of ovulation rate (number of cows with at least 1 corpus luteum on Day 11 after AI as a percentage of the total number of cows in each group), pregnancy rate to first AI (number of pregnant cows after first AI as a percentage of the total number of cows in each group), and pregnancy rate to second AI (number of pregnant cows after 2 rounds of AI – first AI plus return AI – within 30 d of first AI as a percentage of the total number of cows in each group).

Logistic regression analysis was carried out for the dependent variables: ovulation and pregnancy rates in response to first and to second AI. Lactation number, milk production and body condition on day 50 postpartum were considered independent factors and coded as continuous variables. Insemination during the warm period, coded as a dichotomous variable (where 1 means presence and 0 absence), and treatment regime, coded as a class variable (where Ovsynch regime was considered as the reference), were also considered factors in the analysis.

Regression analysis (SAS software, Logistic procedure; 1992) was performed according to the method of Hosmer and Lemeshow (1989). Basically, this method involves five steps as follows: preliminary screening of all variables for univariate associations; construction of a full model, using all the significant variables resulting from the univariate analysis; stepwise removal of nonsignificant variables from the full model and comparison of the reduced model with the previous model for model fit and confounding; evaluation of interactions among variables; and assessment of model fit using Hosmer-Lemeshow statistics. Variables with univariate associations showing P values < 0.25 were included in the initial model. We continued modeling until all the main effects or interaction terms were significant according to the Wald statistic at $P < 0.05$.

Values are expressed as the mean ± standard deviation (SD).

RESULTS

Table 1. Ovulation, return to estrus and pregnancy rates following the treatment regimes Ovsynch and specific synchronization (Ssynch).

Treatment	Number of cows	Ovulation n (%)	Pregnancy to first AI n (%)	Return to estrus n (%)	Pregnancy to second AI n (%)
Ovsynch[a]	205	170 (83)	56 (27)	55 (37)	74 (36)
Ssynch	223	196 (88)	97 (43)	41 (33)	118 (53)
CL subgroup[b]	130	116 (89)	58 (45)	29 (40)	73 (56)
NE subgroup[c]	58	50 (86)	22 (38)	9 (25)	27 (47)
PF subgroup[d]	26	22 (85)	12 (46)	2 (14)	13 (50)
OC subgroup[e]	9	8 (89)	5 (56)	1 (25)	5 (56)

[a]Group Ovsynch = 100 µg GnRH im on Day 0; 500 µg cloprostenol im on Day 7 followed by 100 µg GnRH im 32 h later; AI 16 to 20 h after the second GnRH dose.

[b]Subgroup CL = 500 µg cloprostenol im on Day 0; 250 IU hCG plus 1 mg EB im 12 h later; AI 48 h after cloprostenol treatment.

[c]Subgroup NE = cows inseminated at natural estrus.

[d]Subgroup PF = 1.55 g intravaginal progesterone (PRID) for 9 d; 100 µg GnRH im on Day 0; 500 µg cloprostenol im on Day 7; AI 56 h after PRID removal.

[e]Subgroup OC = 100 µg GnRH plus 500 µg cloprostenol im on Day 0; 500 µg cloprostenol im on Day 14 followed by 100 µg GnRH im 36 h later; AI 24 h after the second GnRH dose.

Table 2. Odds ratios of the variables included in the final logistic regression model for pregnancy rate to first AI.

Factor	Class	n	Odds ratio	95% Confidence Interval		P
Treatment	Ovsynch	205				
	Ssynch	223	2.1	1.39	3.16	<0.0001
Insemination in	0	322				
the warm period	1	106	0.45	0.27	0.74	0.002

Likelihood ratio test = 535.8, 2 d.f., P = 0.001.

Hosmer and Lemeshow Goodness of Fit Statistic = 3.24, 2 d.f., P = 0.2 (the model fits).

Table 3. Odds ratios of the variables included in the final logistic regression model for pregnancy rate to second AI (first AI plus return AI).

Factor	Class	n	Odds ratio	95% Confidence Interval		P
Treatment	Ovsynch	205				
	Ssynch	223	2.1	1.4	3.09	<0.0001
Insemination in	0	322				
the warm period	1	106	0.33	0.2	0.54	<0.0001

Likelihood ratio test = 552, 2 d.f., P = 0.001.

Hosmer and Lemeshow Goodness of Fit Statistic = 3.33, 2 d.f., P = 0.19 (the model fits).

Of the study population of 428 cows, 106 (25%) were first inseminated in the warm and 322 in the cool period. The mean lactation number was 2.4 ± 1.6, ranging from 1 to 11 lactations. On Day 50 postpartum, mean daily milk production was 41.7 ± 9.7 kg, ranging from 20 to 72 kg, and mean body condition was 2.6 ± 0.4 points (range 2 to 3.5 points).

All the animals in the Ssynch group were inseminated within 33 d of starting the protocol. The mean number of days from calving to first AI in the Ovsynch and Synch groups, were 51.2 ± 2.1 (range 48 to 54 d) and 53.5 ± 7 (range 44 to 77 d), respectively. The mean number of days from calving to pregnancy, also respectively for both groups, were 56.8 ± 9.9 (range 48 to 83 d) and 57.2 ± 10.5 (range 44 to 91 d).

Table 1 shows the results recorded for the Ovsynch and Ssynch groups. Logistic regression analyses of ovulation rates indicated no significant effects of the treatment regime. Analyses of pregnancy rates to first or second AI indicated no significant effects of lactation number, milk production and body condition. The variables treatment regime and season were included in both final models (Tables 2 and 3). No significant interactions were found.

DISCUSSION

Our results clearly show an improved response of postpartum presynchronized cows to a specific estrous synchronization protocol applied according to ovarian status, over the response shown by those subjected to a single protocol - Ovsynch in our case - regardless of ovarian status. Cows undergoing specific synchronization were 2.1 times more likely to become pregnant to first and second AI, compared to those synchronized using the Ovsynch protocol. These results suggest that when evaluating estrous synchronization programs by economic decision analysis methods, strategies other than single protocols need to be considered (Nebel and Jobst, 1998).

To relate these findings to those emerging from other clinical trials, factors at the farm level such as the percentage of cyclic cows, the efficiency of estrus detection and the cost of a specific synchronization program should logically be considered. As herd sizes and milk production continue to increase, the incidence of reproductive disorders also appears to be on the increase (Foote, 1996; Butler, 2000). If we consider this factor alone, the cost of a veterinary program performed on a weekly basis could perhaps be recovered owing to prompt diagnosis and treatment of reproductive disorders. Further, veterinary supervision of ovarian status before applying a program of estrous synchronization and timed AI would no doubt notably improve the reproductive performance associated with a systematic breeding program (De Rensis et al., 2002). Under our work conditions, 58 (26%) of the 223 cows included in the specific synchronization group were inseminated at natural estrus. If these cows had not been inseminated, i.e., if estrus had gone unnoticed due to the common practice of reducing the time spent observing cows for estrus, they would have been registered as having a corpus luteum in a subsequent veterinary visit and then synchronized for estrus.

Our therapeutic approach to cows with cysts and inactive ovaries with follicular persistence could explain the benefits of this specific synchronization program. Five out of 9 cows with cysts and 12 out of 26 with persistent follicles became pregnant to first AI. Although, given the design of the present study, it was not possible to establish the percentage of cows with cysts in the Ovsynch group, we previously found (López-Gatius and López-Béjar, 2002) the protocol used here for synchronization plus timed insemination of cows with cysts to be more successful than the Ovsynch procedure when applied at the start of the service period; pregnancy rates for cows with cysts after specific treatment (28.1%) being higher than those subjected to Ovsynch (3.1%). Similarly, in another previous study (López-Gatius et al., 2001), cows with follicular persistence undergoing Ovsynch and time inseminated showed a lower pregnancy rate (4.1%) than those subjected to the current protocol using progesterone, GnRH and PGF2α (34.2%).

There was no significant effect of the treatment regime on ovulation rate. The ovulation rate was indeed high in both groups (83% Ovsynch, 88% Ssynch) and was similar to the rate observed in the subset of cows inseminated at natural estrus (NE subgroup 86%). Thus, in the Ovsynch group, the high ovulation rate yet relatively lower pregnancy rate to first AI, could reflect asynchrony between the time of insemination and ovulation. It was recently demonstrated (Peters and Pursley, 2002; López-Gatius et al.,2003) that presynchronization during the preservice period increases the subsequent proportion of cows with luteal function, but the pregnancy rate to timed AI does not improve when Ovsynch is applied to presynchronized cows (Peters and Pursley, 2002). Our results suggest that a specific estrous synchronization protocol applied according to the ovarian status of the cows is able to make better use of the beneficial effects of presynchronization on ovarian function, compared to the Ovsynch protocol.

The insemination season was a significant risk factor for pregnancy in response to first or second AI. Cows inseminated in the warm period were 2.2 times (1/0.45) less likely to become pregnant to first AI and 3 times (1/0.33) to second AI, compared to those inseminated in the cool season. These findings are consistent with those emerging from studies performed in our geographical area, in which it was noted that a cool environment was related to preserved fertility and a reduced risk of reproductive disorders (López-Gatius et al., 2002a, b; López-Gatius, 2003).

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VIII. GENERAL DISCUSSION

1. Is presynchronization a good management toll in improving reproductive performance in postpartum dairy cows?

Delayed resumption of normal ovarian activity following parturition, poor estrous detection rate, silent estrus, improper timing of insemination and ovarian disorders during early postpartum period are the major problems, which decrease the reproductive efficiency in a dairy farm (Barr, 1975; Boyd, 1977; Bailie, 1982). Our research was focused to reduce the influence of these factors to a maximum extent so as to improve the reproductive efficiency in postpartum dairy cows.

The sequential changes that take place from parturition to subsequent conception in lactating dairy cows are complex (Butler and Smith, 1989). Following calving, several ovarian disorders that cause reduction in reproductive efficiency of postpartum dairy cows were reported (Opsomer et al., 2000) Among these, a prolonged luteal activity phase subsequent to resumption of ovarian activity in the preservice period was one of the major disorders reported (Opsomer et al., 1998; 2000; Smith and Wallace, 1998). Prolonged luteal phases being reported as major disorder is due to the inability of the uterus, suffering from uterine abnormalities to produce prostaglandin, which prolongs the intercalving period (Farin et al., 1989). It has been demonstrated that puerperal disturbances dramatically retard uterine involution (El-Din Zain et al., 1995). Prolonged luteal phases were also reported without any clinically observable cause (Bulman and Lamming, 1977). The cause for prolonged luteal phase in the present investigation could be due to subclinical uterine infection. Effectiveness of prostaglandin treatment on postpartum uterine disorders have been reported in cattle (Wenzel, 1991).

The incidence of metritis-pyometra complex is significantly higher in the control group suggesting that presynchronization reduces this uterine disorder in postpartum cows. This result concurs with the results of Olson et al. (1983) who reported the successful treatment of pyometra condition in postpartum dairy cows with prostaglandin treatment, as in this condition, the ovary will be bearing a corpus luteum susceptible to prostaglandin. There is a significant reduction in ovarian cysts in the treatment group when compared with control group and this may be due to the recovery of cows with luteal cysts, as luteal cysts are usually responsive to prostaglandin treatment (Nanda et al., 1988). However, presynchronization treatment fails to reduce the incidence of persistent follicles in early postpartum dairy cows. In cows with persistent follicles, López-Gatius et al. (2001) observed a poor response to GnRH - Prostaglandin treatment.

Presynchronization treatment enhanced the percentage of cows with the presence of luteal activity, suggesting that presynchronization would increase the number of cows that respond to subsequent estrous synchronization programs when compared to cows without presynchronization treatment.

In any reproductive management program, the ultimate aim is to achieve a higher pregnancy rates in dairy cows. In this study, a two-fold increase in pregnancy rate was recorded in presynchronized group when compared to the control group. This fact demonstrates the effectiveness of presynchronization in early postpartum dairy cows.

The increase in pregnancy rates in presynchronized group of cows could be attributed to the higher percentage of cows with luteal activity and the decrease in percentage of cows with ovarian cysts and metritis-pyometra complex following presynchronization treatment.

Increase in pregnancy rates in presynchronized cows could also be due to induction of estrus following prostaglandin treatment. In postpartum dairy cows, occurrence of estrous cycles in the first 60 days postpartum has been associated with increased conception rates (Thatcher and Wilcox, 1973). Initiation of cyclic activity, with the completion of some estrous cycles before insemination, seems to the key factor in enabling cows to maintain a high reproductive rate (Butler and Smith, 1989). Each estrous cycle is accompanied by estrogen secretion followed by the secretion of progesterone. Estrogens stimulate blood flow to the uterus, uterine contractions, and initiate leukocytic invasion of the uterus, thus facilitate the removal of any debris that remain from the previous parturition. Progesterone, stimulate endometrial gland growth, prepare the uterus to receive and nourish a new embryo (Foote and Riek, 1999).

Finally, we can conclude that presynchronization helps to improve the reproductive performance in early postpartum dairy cows. However, the effect of presynchronization on cows with persistent follicles and ovarian cysts, possibly follicular cysts is insignificant.

2. Is evaluation of ovarian activity essential before the commencement of timed insemination estrous synchronization protocol in early postpartum dairy cows?

In experiment 2, all the cows before the initiation of estrous synchronization treatment were presynchronized with two doses of PGF2α to hasten involution of postpartum uterus and to reduce subclinical uterine disorders (Paisley et al., 1986) that could interfere with results. In early postpartum dairy cows, anestrus, attributable to an anovulatory condition, is one of the most frequent disorders (Opsomer et al., 2000; Wiltbank et al., 2002). In dairy cows showing no luteal activity due to presence of anovulatory follicles, progesterone based GnRH-PGF2α estrous synchronization protocol was reported to be more successful than Ovsynch protocol (López-Gatius et al., 2001). In this recent timed insemination estrous synchronization protocol, progesterone was administered for 9 d and GnRH was given on Day 0 and PGF2α on Day 7. Reports regarding high incidence of anovulatory conditions during early postpartum period (Wiltbank et al., 2002), has made us to believe that this recently described progesterone based estrous synchronization protocol could be more advantageous than Ovsynch in early postpartum dairy cows.

Two progesterone based timed insemination estrous synchronization protocols, one with and another without inclusion of second dose of GnRH after removal of PRID (9 d progesterone treatment) were compared with Ovsynch protocol in presynchronized lactating dairy cows. Overall analysis of the data show significant increase in ovulation rate in progesterone based protocol that included the second dose of GnRH after removal of PRID. However, pregnancy rates between the three treatment groups were found to be similar. In fact, the second GnRH given at the time of preovulatory LH surge improved the ovulation rate in progesterone based estrous synchronization protocol. This favorable effect of the second dose of GnRH could be attributed to an amplified spontaneous endogenous preovulatory LH surge by the second GnRH dose administered around the time of the endogenous LH surge (Lucy and Stevenson, 1986; Rosenberg et al., 1991).

The interesting and significant findings were noted when the data were stratified and analyzed based on presence of low (with no luteal activity; P4 < 1ng/mL) or high progesterone concentrations (with luteal activity; P4 ≥ 1ng/mL) at the time of the onset of treatment. Significant difference in pregnancy and ovulation rates were reported between cows with or without luteal activity at the onset of estrous synchronization treatment. Progesterone based treatments significantly improve the pregnancy and ovulation rates than

Ovsynch protocol in cows with low progesterone concentration at the initiation of estrous synchronization treatment. Whereas, it is the other way around in case of cows with high plasma progesterone concentration at the onset of treatment.

Following the second dose of PGF2α (presynchronization treatment), cyclic cows with PGF2α responsive corpus luteum at the time of presynchronization, would have responded to PGF2α and would have returned to estrus. Onset of estrus following PGF2α depends upon the stage of estrous cycle at the time of PGF2α treatment and in 90 % of cows it falls between Day 2 to Day 6 of PGF2α treatment (Wenzel, 1991). In all cows, estrous synchronization treatment was started on Day 7, after the second dose of PGF2α used for presynchronization treatment. Hence, cows that came to estrus later following to second dose of PGF2α used in presynchronization would be in metestrum (low progesterone concentration group) and cows that came to estrus earlier would be in early luteal phase (high progesterone concentration group) on Day 7 after the second dose of PGF2α, that is on day of initiation of estrous synchronization treatment.

To achieve higher pregnancy rates, early luteal phase was reported to be most favorable period for initiation of Ovsynch when compared to initiation of Ovsynch during metestrum or late diestrum (Vasconcelos et al., 1999; Moreira et al., 2000a). Thus, cyclic dairy cows in the low progesterone concentration group (metestrum period) at the time of initiation of Ovsynch protocol result in poor pregnancy rates. An additional cause for the poor pregnancy rate following Ovsynch treatment in cows with low progesterone concentration could be due to anovulatory condition (López-Gatius et al., 2001; Wiltbank et al., 2002). Most anovulatory cows probably lack GnRH responsive follicles at the time of the first GnRH dose of the Ovsynch protocol. It has been shown that ovulation of the follicle in response to the first GnRH injection is a prerequisite for the success of the Ovsynch program (Vasconcelos et al., 1999). Moreover, there was limited response to treatment with GnRH plus PGF2α in cows with a persistent follicle (López-Gatius et al., 2001).

Obviously, in cyclic dairy cows with high progesterone concentration at the onset of Ovsynch protocol, a higher pregnancy rate was obtained, as the Ovsynch treatment was initiated during early luteal phase of the estrous cycle. Improved pregnancy rate after timed insemination recorded in the Ovsynch-high progesterone group concurs with the results of previous studies in which sequential treatment with GnRH and PGF2α was shown to synchronize follicular development and the regression of the corpus luteum in dairy cows (Burke et al., 1996; Schmitt et al., 1996b; Pursley et al., 1997a,b).

In contrast, progesterone based estrous synchronization protocol produced higher ovulation and pregnancy rates in cows with low progesterone concentration at the time of treatment onset. This shows that the progesterone treatment regime seems to be sufficient to facilitate follicular growth and maturation before ovulation after the removal of the progesterone device, leading to improved ovulation and pregnancy as reported by Stevenson et al. (1997) in anestrous beef cows. However, lower pregnancy and ovulation rates were achieved when the same treatment was applied to cows showing high progesterone concentration at the time of onset of treatment.

Thus, the results of trail 2 show that evaluating ovarian activity by means of plasma progesterone assay before initiation of estrous synchronization and adopting appropriate timed insemination estrous synchronization protocol according to ovarian activity of the cows will no doubt improve the reproductive performance of early postpartum dairy cows.

3. Is application of specific timed estrous synchronization protocol on the basis of ovarian status better than applying Ovsynch protocol irrespective of ovarian status, in the improvement of reproductive performance in dairy cows?

The resumption of normal ovarian cyclic activity is one of the most important criterion for achieving better reproductive performance in postpartum dairy cows. However, regular cyclicity after 50 days postpartum was observed in only 51% of all high producing dairy cows examined (Opsomer et al., 2000). The high incidence of ovarian disorders in the preservice/postpartum period (Lamming and Darwash, 1998; Opsomer et al., 1998, 2000) appears to be one of the reasons for the decrease in reproductive efficiency in early postpartum dairy cows (Kesler and Garverick, 1982).

After an appreciable advancement in controlling ovulation within 8 h for fixed timed insemination in dairy cows with normal ovarian activity (Pursley et al., 1995), the current trend of veterinary reproductive physiologists is focused towards the development of estrous synchronization protocols for postpartum dairy cows with ovarian disorders to control ovulation to permit fixed timed insemination (López-Gatius et al., 2001; Pursley et al., 2001; López-Gatius and López-Béjar, 2002).

More recently, timed insemination estrous synchronization protocols for ovarian cysts and anestrus due to persistent follicle have been developed and it was demonstrated to be more effective than Ovsynch protocol in postpartum dairy cows (López-Gatius et al., 2001; López-Gatius and López-Béjar, 2002). The authors clearly described the mechanism by which the specific timed insemination estrous synchronization protocol found to be successful in ovarian disorders.

For postpartum dairy cows with mature corpus luteum, López-Gatius (2000a), demonstrated that the administration of prostaglandin followed by 250 IU hCG and 1 mg estradiol benzoate 12 h later, was effective for the synchronization of estrus, followed by timed AI.

The above findings regarding the incidence of ovarian disorders during early postpartum periods and the recent report of specific timed insemination estrous synchronization protocols for different ovarian conditions has made us to come to an opinion that the specific synchronization of estrus according to the ovarian status of the cows (Specific-Synch) would lead to an enhanced improvement of reproductive performance in early postpartum dairy cows when compared to Ovsynch protocol to all cows irrespective of ovarian status.

Thus in trail 3, all the dairy cows were presynchronized with 2 prostaglandin injections given 14 d apart, starting from Day 14 to 20 postpartum. The cows were then assigned to 1 of 2 treatment groups. The first group of cows was treated with Ovsynch protocol without the prior knowledge of ovarian status. The cows in the second group (Specific-Synch) were weekly subjected to transrectal ultrasound examinations for 4 weeks, or until AI or till the start of treatment, and were divided into four subgroups (1.Cows with a corpus luteum 2. Cows inseminated at natural estrus; 3. Cows with persistent follicle and, 4. Cows with ovarian cysts) according to their ovarian status and, specific breeding program was adopted accordingly.

Results from trail 3 clearly demonstrate a distinct improvement in reproductive performance in presynchronized early postpartum dairy cows in response to a specific estrous synchronization protocol applied according to ovarian status, over the response shown by those cows subjected to Ovsynch protocol without prior knowledge of ovarian status. Since Specific–Synch breeding program enhances the reproductive performance of dairy cows, the program seems to be cost effective even though it requires weekly veterinary visits.

Furthermore, this finding obviously shows that gynecological examination of ovarian status, during weekly veterinary visits, before deciding the appropriate breeding program, will augment the reproductive performance in postpartum dairy cows and thereby increase the profitability of the dairy farm. Indeed, the role of veterinarian will be quintessential for improving reproductive performance in postpartum dairy cows and in the profitability of a dairy farm.

IX. CONCLUSIONS

1. Presynchronization during the preservice period using 2 prostaglandin doses given 14 d apart, improves ovarian activity from Days 50 to 71 postpartum, and increases the number of cows that enter into estrus, which in turn ovulate and become pregnant.

2. Luteal activity at the time of onset of timed insemination estrous synchronization protocol influences subsequent reproductive performances in lactating dairy cows and therefore:

   a. Applying Ovsynch protocol for cows with high progesterone concentration ($\geq$1 ng/mL), and progesterone – GnRH – PGF2α treatment for cows with low progesterone concentration ($<$1ng/mL) will improve the overall reproductive performance of the dairy farm.

3. Administration of progesterone treatment for 9 d between GnRH and PGF2α in GnRH-PGF2α-GnRH protocol is not found to improve the reproductive performance in the general population of presynchronized dairy cows when compared to the Ovsynch protocol.

4. A specific estrous synchronization protocol applied according to the ovarian status of the cows markedly improves the reproductive performance in early postpartum dairy cows when compared to cows treated with Ovsynch protocol without the assessment of the ovarian status at the time of initiation of treatment.

APPLIED ASPECTS

The overall salient findings of the present investigation recommend the following to improve the reproductive performance in early postpartum dairy cows:

1. **Presynchronization of all early postpartum dairy cows with two doses of PGF2α at 14 d apart before initiation of fixed timed estrous synchronization protocols.**

2. **Administration of progesterone for 9 d is recommended between GnRH and PGF2α in GnRH-PGF2α-GnRH protocol in the case of early postpartum dairy cows with low progesterone concentration (no luteal activity) at the time of onset of treatment.**

3. **Gynaecological examination of early postpartum dairy cows for ovarian status and the application of suitable fixed timed estrous synchronization protocols instead of implementing Ovsynch protocol to all postpartum dairy cows without any gynaecological examination by the veterinarian.**

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