OVARIAN FOLLICULAR DYNAMICS, LH PROFILES, CORPUS LUTEUM FUNCTION AND PREGNANCY FOLLOWING TWO OVULATION SYNCHRONIZATION/TIMED ARTIFICIAL INSEMINATION PROTOCOLS IN CATTLE

by

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ABSTRACT

The reduced fertility following earlier estrus synchronization protocols made it necessary to develop new protocols based on our understanding of ovarian follicular dynamics and corpus luteum (CL) function in cattle. "Ovsynch" and "CIDR" are emerging protocols for ovulation synchronization that would facilitate artificial insemination at a fixed time (TAI) without estrus detection. Unfortunately, the limited and available literature on Ovsynch and CIDR treatment protocols focuses mainly on ovarian follicular dynamics during treatment and ovulation synchronization success. Therefore, the objectives of this study were to compare LH profiles, follicular dynamics, CL function and pregnancy rates (PR), following treatment with Ovsynch and CIDR ovulation synchronization/TAI protocols. In the first experiment, ultrasonography was performed throughout the treatment protocols and following ovulation for one complete cycle to monitor ovarian follicular and CL dynamics. Serial blood samples were taken to determine the LH profile and progesterone (P_4) concentrations throughout the treatment protocols and for one complete cycle following ovulation. Prolonged duration and larger diameter of the ovulatory follicle in the Ovsynch compared to CIDR protocol did not affect post synchronization LH profiles, follicular dynamics and P_4 concentrations, which were similar between Ovsynch and CIDR ovulation synchronization protocols. In experiment 2, CL's formed after Ovsynch and CIDR ovulation synchronization protocols were incubated with four different hormone treatments; LH, $PGF_{2\alpha}$, LH + $PGF_{2\alpha}$, and control (no treatment) to determine in vitro P_4 secretion. Corpora lutea formed after Ovsynch and CIDR ovulation synchronization protocols yielded similar in vitro P_4 concentrations at different stages of the synchronized cycle. The response to LH, $PGF_{2\alpha}$,

and LH + $PGF_{2\alpha}$ was also not different between the two ovulation synchronization protocols. In experiment 3, the focus was to compare in vivo P_4 profiles and PR in lactating dairy cows following treatment with the Ovsynch and CIDR ovulation synchronization/TAI protocols. In vivo P_4 production and PR were similar between the Ovsynch and CIDR treatment protocols. Similar post synchronization LH profiles, follicular dynamics, P_4 profiles and PR following Ovsynch and CIDR ovulation synchronization protocols suggest that both protocols can be equally effective in synchronization of ovulation, elimination of estrus detection and enhancement of pregnancy in heifers as well as in cows. However, based on frequency of handling, cost, and industry approval, this study favors the use of the Ovsynch over the CIDR for ovulation synchronization in cows and heifers.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

°C	= degrees Centigrade
µg	= microgram
µl	= microlitre
µm	= micrometer
3βHSD	= 3β hydroxy steroid dehydrogenase
AI	= Artificial insemination
BSA	= bovine serum albumen
cAMP	= cyclic adenosine monophosphate
CAP	= chloromadionone acetate
CIDR-B®	= controlled internal drug release
CL	= corpus luteum
CR	= conception rate
DAG	= 1, 2-diacylglycerol
DF	= dominant follicle
DNA	= deoxyribonucleic acid
E_2	= estradiol-17β
EB	= estradiol benzoate
ECP	= estradiol cypionate
ET-1	= endothelin-1
FP	= mRNA for $PGF_{2\alpha}$ receptors
FSH	= follicle stimulating hormone
g	= gram

g = gravity

GDP = guanosine diphosphate

GnRH = gonadotropin releasing hormone

GTP = guanosine triphosphate

h = hour(s)

HDL = high density lipoprotein

HPO = hypothalamo-pituitary-ovarian axis

hCG = human chorionic gonadotropin

i.m. = intramuscular

IGF-1 = insulin-like growth factor

IGFBP = IGF-binding protein

INF-t = interferon tau

IP_3 = inositol-1,4,5-triphosphate

JMP® = JMP IN statistical package

LDL = low density lipoprotein

LH = luteinizing hormone

LLC = large luteal cells

MAP = medroxy progesterone

mg = milligram

Mg^{+2} = magnesium

MGA = melengestrol acetate

ml = millilitre

mm = millimeter

mRNA = messenger ribonucleic acid

n = sample size or animal numbers

ng = nanogram(s)

P_4 = progesterone

PBS = phosphate buffered saline

PGE_2 = prostaglandin E_2

$PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$

PGHS-2 = prostaglandin G/H synthase

PIP_2 = phosphatidyl inositol 4,5-bisphosphate

PKA = protein kinase A

PKC = protein kinase C

PR = pregnancy rate(s)

PRID = progesterone releasing intravaginal device

SAS = statistical analysis system

SD = standard deviation

SEM = standard error of the mean

SF = subordinate follicle

SLC = small luteal cells

SMB = synchromate-B

StAR = steroidogenic acute regulatory protein

TAI = timed artificial insemination

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DEDICATION

I dedicate this thesis to my late father. This thesis is also dedicated to my mother, who had to work hard since I was seven years old and without her sacrifices and hardships, this thesis would no be existent. Finally, the thesis must be dedicated to my partner, love and the mother of my children, my wife Alia. I have no clue where I would be had it not been for her constant and relentless encouragement's.

"Quitters don't win and winners don't quit". I chose to be a winner.

CHAPTER 1

GENERAL INTRODUCTION

Reproductive efficiency in cattle (beef and dairy herds) has a marked influence on profitability. The calving to first service interval for individual cows is a significant factor in determining the calving index of a herd (David et al., 1971), and the efficiency with which cows are submitted for their first service is therefore a key element in herd fertility management. A calving interval of 12 to 13 months has been recommended for dairy as well as beef cows (Lucy et al., 1986; Duffy et al., 2000). Approximately 30-50% of beef cows in the United States fail to produce a calf in less than 365 days (Williams, 1990). Further, dairy heifers must be bred at 14 to 15 months of age to achieve the North American calving target age of 24 months (Ambrose et al., 2002). An integral component in achieving the desired calving interval in cows as well as the targeted breeding age in heifers, is the incorporation of efficient and accurate estrus detection, proper semen handling techniques, and timed artificial insemination (TAI) relative to ovulation.

Pregnancy rate (PR) after AI is the product of estrus detection and conception rates (Burke et al., 1996), and it can be improved by maximizing estrus detection rates. The use of AI has not been widely adopted by beef producers because estrus detection is time consuming (Small et al., 2001). On the other hand, approximately 70 to 90% of dairy farms in North America use AI as means of breeding (Rajamahendran et al., 1993; Pursley et al., 1997b). However, increases in herd size and milk production have led to

inefficient and inaccurate estrus detection, a major problem that leads to adverse reproductive efficiency and economic losses (Jobst et al., 2000). Estrus detection rate has been estimated to be 50% in dairy cows (Rajamahendran et al., 1993; De Rensis and Peters, 1999). Failure to detect estrus accurately has been calculated to cost the US dairy industry $300 million per year (De Rensis and Peters, 1999). Artificially inseminating an animal that is not in estrus (when the milk progesterone concentration is greater than 1 ng/ml) may occur in 10 to 12% of all services (Pennington et al., 1985) and may be as high as 20 to 30% under certain management conditions (Senger et al., 1988; Rajamahendran et al., 1993). Under these circumstances, not only are the costs of maintaining the cow and purchasing and holding semen wasted, but other reproductive problems, including early embryonic mortality (Claus et al., 1983), may result from wrongly timed inseminations. Thus, maximizing estrus detection can improve overall reproductive efficiency in dairy cattle.

Hormone treatments resulting in estrus synchronization have greatly improved the reproductive management of cattle since they enable producers to predict and thus manage the onset of estrus and ovulation. They also improve the use of AI programs since they reduce the time and labor for detection of estrus. Implementation of estrus synchronization protocols may therefore help improve production efficiency of cattle herds resulting in improved economic status for the producer.

Initial attempts to synchronize the onset of estrus focused on manipulating the length of the estrous cycle; for example, progestagens were used to extend the luteal

phase of the estrous cycle (Stock and Fortune, 1993; Rajamahendran and Manikkam, 1994) and prostaglandins were used to shorten the luteal phase of the estrous cycle (Lucy et al., 1986; Stephens and Rajamahendran, 1998). However, considerable variation in the maturation process of the dominant follicle (DF) and subsequent ovulation between animals resulted in poor estrus synchronization. This in turn resulted in reduced fertility rates making it necessary to identify a precise method(s) of inducing and synchronizing estrus and ovulation.

An increase in the basic understanding of ovarian follicular and corpus luteum (CL) dynamics in cattle (Rajamahendran et al., 1994) combined with the development of treatment regimes to manipulate these two processes over the last decade have resulted in development of new ovulation synchronization protocols; namely Ovsynch (Pursley et al., 1995; Hirad et al., 1999) and CIDR + estradiol (Day et al., 2000; Martinez et al., 2000). These two protocols, are both based on: a) elimination of the DF, b) initiation of a new follicular wave, and c) synchronization of ovulation and timed artificial insemination (TAI). The Ovsynch protocol is composed of 1) an initial injection of gonadotropin-releasing hormone (GnRH) to induce ovulation of the DF, followed by the initiation of a new follicular wave, which either yields a new CL or enhances the function of the already present CL (Macmillan and Thatcher, 1991; Milvae et al., 1984; Rajamahendran et al., 2001); 2) a prostaglandin- $F_{2\alpha}$ ($PGF_{2\alpha}$) injection is given seven days later to cause luteal regression of the existing or induced CL; and 3) a second GnRH injection is given 48 h later to synchronize ovulation of the newly formed DF, following regression of the CL. The CIDR protocol uses: 1) an initial injection of progesterone (P_4) and estradiol and an

insertion of controlled internal drug release (CIDR-B®; progesterone device) to synchronize follicular wave emergence 3-5 days after estradiol injection, and block luteinizing hormone (LH) surge and ovulation (Martinez et al., 2000; Rajamahendran et al., 2001), 2) a $PGF_{2\alpha}$ injection (7 days later) to cause luteal regression of existing or induced CL, and 3) a second estradiol injection after removal of CIDR to synchronize ovulation (Hanlon et al., 1997; Lammoglia et al., 1998; Martinez et al., 2000).

Pregnancy rates following Ovsynch and fixed TAI have averaged about 40% in lactating dairy cows (Ambrose et al., 1999; Ambrose et al., 2000), and dairy heifers (Stevenson et al., 2000). Interestingly, the 40% PR in heifers is considerably lower than what could be achieved with breeding at detected estrus (≤ 70%; Pursley et al., 1997b). Using the Ovsynch protocol, incidences of premature ovulation (before the second GnRH injection) have been reported by different researchers (Pursley et al., 1995; Vasconcelos et al., 1999; Moreira et al., 2000). In contrast, PR following CIDR and fixed TAI have averaged 60-80% in beef heifers and cows (Martinez et al., 2000) as well as in lactating dairy cows (Burke et al., 2000; Day et al., 2000). The CIDR-B® device has Canadian approval for synchronization in beef and dairy, with the exception of lactating dairy cows. Although the efficacy of the CIDR device in synchronization of estrus has been examined (Macmillan and Peterson, 1993; Lucy et al., 2001), there is little information available on the efficacy of CIDR-based protocols for the synchronization of ovulation and TAI.

High ovulation response regardless of the stage of the cycle at which treatment is

initiated, follicular dynamics, LH profiles, close synchrony in time of ovulation, adequate CL function, acceptable fertility at the synchronized ovulation, are some of the recommended features in designing an ovulation synchronization program for cattle. In addition, minimal handling of cattle, and cost-effectiveness are extra features that are to be considered when designing and applying ovulation synchronization protocols that are based on TAI. Unfortunately, the limited and available literature on Ovsynch and CIDR treatment protocols focuses mainly on ovarian follicular dynamics during treatment and ovulation synchronization success (Vasconcelos et al., 1999; Moreira et al., 2000; Bo et al., 2000; Day et al., 2000). Clearly, further research is warranted to understand LH responses, CL formation and function, ovarian follicular dynamics, and PR following Ovsynch and CIDR treatment protocols for ovulation synchronization. Therefore, the objectives of this study are to compare LH profiles, follicular dynamics, CL function, and PR following the Ovsynch and CIDR ovulation synchronization and TAI protocols.

CHAPTER 2

LITERATURE REVIEW

This chapter provides background information on hormonal control of the estrous cycle, ovarian follicular dynamics in cattle, corpus luteum (CL) development, function, and its demise. Current status of estrus synchronization protocols in cattle, estrus synchronization protocols in relation to elimination of the dominant follicle (DF) and induction of new follicular wave, protocols in relation to emergence of new follicular wave and synchronization of ovulation and timed artificial insemination (TAI) will also be reviewed. Finally, the rationale and objectives of the thesis are presented.

I. Estrous cycle in the bovine

The interval between two estrus periods is called the estrous cycle. The cow is a polyestrous animal exhibiting regular estrous cycles every 21 days, when not pregnant (Hansel and Echternkamp, 1972). The estrous cycle is divided into the follicular phase, and the luteal phase with the former being approximately 3-4 days and the latter approximately 17 days in length. The estrous cycle can also be broken down into four designated periods: proestrus, estrus, metestrus and diestrus (Figure 2.1; Sorensen, 1979). Proestrus (before desire) is the period preceding estrus (from days 17-21) when the animal is in a state of excitement as it prepares for mating. The estrus (desire) period lasts approximately 12-26 h (Gomes, 1978) and corresponds to the time when the animal is very excited and ready for mating. This period is accompanied by an luteinizing hormone

(LH) surge that occurs about 24 h after the estradio-17β (E_2) surge, with ovulation occurring approximately 30 h thereafter (Walters and Schallenberger, 1984; Rajamahendran et al., 1989). Metestrus (after desire) lasts between 2-4 days and is the period when ovulation occurs. During this period the animal recovers from the excitement of mating and begins preparation for pregnancy (Sorensen, 1979). The diestrus (without desire) period can be between days 5-16 and corresponds to the time when the animal is in between estrus periods (Sorensen, 1979).

2.1. Hormonal control of the estrous cycle

The hypothalamo-pituitary-ovarian axis (HPO axis) and the hormones produced at each level of the axis are responsible for the morphological events as well as the behavioral changes, which occur during the estrous cycle (Figure. 2.1). From their respective origin of the HPO axis, both positive and negative feedback mechanisms by the pituitary and the ovary control the secretion of these hormones (Figure. 2.2).

2.1.1. Hypothalamic GnRH

The main neuroendocrine reproductive hormone secreted by the hypothalamus is gonadotropin-releasing hormone (GnRH). This hormone is a decapeptide (10 amino acids) with a sequence of pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2, and regulates the two main reproductive hormones from the gonadotrope cells of the anterior pituitary, follicle stimulating hormone (FSH) and LH (Thatcher et al., 1993). Gonadotropin-releasing hormone is released in a pulsatile pattern occurring at 1 to 3 h intervals (King, 1993), which in turn influences the pulsatile pattern of both FSH and LH

from the gonadotrope cells. The secretion and function of GnRH is regulated by the pituitary (short loop), progesterone (P_4) and E_2 from the ovary (long loop) and by its own receptors by a way of auto-regulation (ultra short loop) (Gregg et al, 1990; Ortmann et al., 1995). The concentration of GnRH starts to increase in proestrus, reaches a peak at estrus, decreases at the end of metestrus, and reaches basal during diestrus (Sorensen, 1979).

2.1.2. Pituitary FSH and LH

In response to pulsatile GnRH, gonadotropes secrete FSH and LH in a pulsatile pattern resulting in the stimulation of steroidogenesis in the ovary (Hillier et al., 1995). Follicle stimulating hormone is responsible for the growth of follicles on the ovary and production of E_2, by binding its receptors on the granulosa cells (Fortune et al., 1988). Significant increases of FSH occur during the estrous cycle; 1) around the time of preovulatory LH surge, 2) 12-24 h after the LH peak coinciding with the time of emergence of the first follicular wave and 3) before the initiation of second or third follicular waves (Ireland et al., 2000). The concentration of FSH has been found to decline on days 2 to 3 of the estrous cycle, even before E_2 rises in blood on days 5 and 8 (Cook et al., 1997; Roche et al., 1998). This indicates that factors such as inhibins/follistatin besides E_2 could regulate FSH secretion (Roche et al., 1998).

Significant increases in LH occur at the time when P_4 concentrations decline below 1 ng/ml at the end of the estrous cycle reaching peak at the beginning of estrus (Hansel and Echternkamp, 1972). The peak of the LH surge lasts between 8 and 10 h

(Hansel and Echternkamp, 1972; Chenault et al., 1975) and ovulation occurs 25-30 hours after the preovulatory surge of LH (Henricks et al., 1970), or 22 h after the LH peak has been attained (Rajamahendran et al., 1989). Plasma concentration of LH increases between days 2 and 4 around the time of onset of P_4 production (Hansel and Echternkamp, 1972), and at the time of DF selection during follicular waves of the estrous cycle (Ireland et al., 2000). In a natural estrous cycle, the breakdown of the germinal vesicle, completion of meiosis I, extrusion of the first polar body, and formation of the secondary oocyte occur about 8 h after the onset of LH surge (King, 1993). The secondary oocyte is then arrested in metaphase II, which occurs 21 h after the onset of LH surge or 8 to 9 h before fertilization and the completion of meiosis II (King, 1993). Thus, LH is involved in the production of E_2 in conjunction with FSH, responsible for the maturation of the oocyte, and rupture of the follicle wall and ovulation of the oocyte. Luteinizing hormone receptors are located on the theca cells of the follicle, where after binding to its receptors, LH stimulates the production of androgens (Fortune and Quirk, 1988). When the follicle reaches 8 or 9 mm in diameter (around time of DF selection), LH receptors start to localize on the granulosa cells (Xu et al., 1995; Beg et al., 2001).

2.1.3. Estradiol-17β (E_2)

Estradiol-17β, mainly secreted from the DF increases during proestrus. The ovulatory DF secretes E_2 during proestrus in sufficient amounts to stimulate the hypothalamus to increase both the frequency and amplitude of GnRH (Hansel and Echternkamp, 1972). This in turn results in increased frequency and amplitude of LH and FSH pulses, which complete follicular maturation and result in a surge of E_2 (Walters and

Schallenberger, 1984). Estradiol-17β starts to increase four days before the last day of the estrous cycle (21 day cycle) reaching peak at time of estrus (Hansel and Echternkamp, 1972). Estradiol-17β declines after the LH surge reaching basal levels during the mid-luteal phase of the estrous cycle (Hansel and Echternkamp, 1972; Walters and Schallenberger, 1984). However, a mid-cycle rise of E_2 can also be detected (Sorensen, 1979), which corresponds either with follicular development (second follicular wave) or to the short cycles reported and is probably high enough in some cows to result in estrus (Macmillan and Watson, 1971).

2.1.4. Progesterone (P_4)

Progesterone concentrations are present at basal levels (< 0.25 ng/ml) on days 1 and 2 of the estrous cycle (Hansel and Echternkamp, 1972). Concentrations begin to increase on days 3 to 12 of the estrous cycle and then stabilize until day 16 of the estrous cycle after which they rapidly decrease following regression of the CL (Hansel and Echternkamp, 1972; Rajamahendran et al., 1976). The presence of high P_4 concentrations blocks the E_2-induced gonadotropin surge in heifers (Kesner et al., 1981). Progesterone acts at the sites of hypothalamus and pituitary (Niswender et al., 2000) and negatively affects LH frequency and concentration. It also inhibits GnRH surges from the hypothalamus, and decreases the number of GnRH receptors by down-regulating mRNA encoding the receptor for GnRH (Niswender et al., 2000). The expression of genes encoding the α as well as β subunits of LH and FSH can also be down regulated by high concentrations of P_4 (Niswender et al., 2000).

2.1.5. Prostaglandin-$F_{2\alpha}$ ($PGF_{2\alpha}$)

Uterine $PGF_{2\alpha}$ is the main factor responsible for luteal regression and cessation of P_4 production in cattle (Knickerbocker et al., 1988). In the case of non-pregnant cattle, the regression of the CL occurs between days 16 and 19 of the estrous cycle (Ginther et al., 1989a; King, 1993).

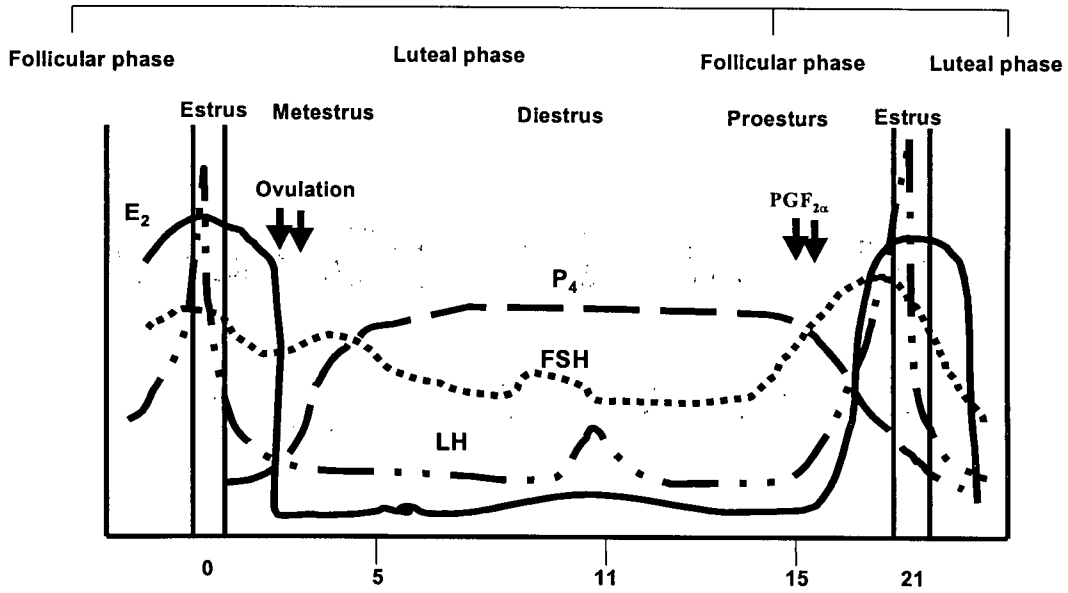

Figure 2.1. Schematic representation of the estrous cycle in the bovine based on 21-day cycle. The cycle is divided into the follicular phase and the luteal phase and defined by four designated periods: proestrus, estrus, metestrus and diestrus. During proestrus (follicular phase), the preovulatory follicle secretes increasing amounts of estradiol-17β. At the onset of estrus, peak levels of estradiol-17β trigger a surge of LH that causes ovulation to occur about 10 to 12 h after the end of estrus. During estrus, high estradiol-17β levels are accompanied with LH and FSH surges with progesterone levels remaining basal. Metestrus period is when ovulation occurs and both LH and estradiol-17β are basal; however, during this stage another FSH surge occurs (necessary for recruitment of follicles) and progesterone starts to increase. During diestrus, LH, FSH and estradiol-17β are basal and progesterone is at its peak. However, surges of LH, FSH and estradiol-17β are detected during mid-luteal phase of the estrous cycle. The surges of FSH and estradiol-17β are associated with emergence of the second follicular wave and development of the second wave dominant follicle, respectively. In a non-pregnant cow, $PGF_{2\alpha}$ from the uterus acts on the CL and causes it to regress. (Adapted from Sorensen, 1979).

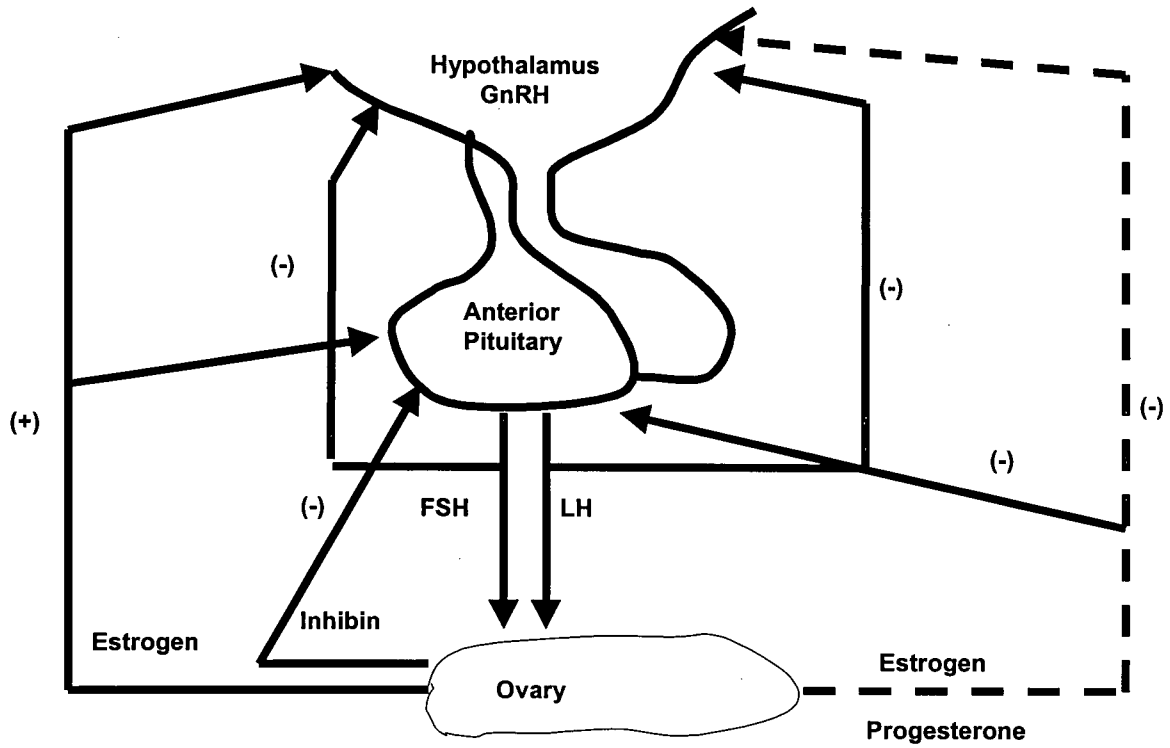

Figure 2.2. Schematic representation of the hypothalamo-pituitary-ovarian axis (HPO axis). The synthesis and release of hypothalamic hormones are regulated by both pituitary and steroid hormones through two feedback mechanisms: a long and a short loop. Long feedback involves interaction among the ovary (estradiol-17β and progesterone), pituitary (LH and FSH) and hypothalamus (GnRH). In the short feedback system, the levels of pituitary gonadotropins (LH and FSH) can influence the secretory activity of the releasing hormones without mediation of the ovary. A feedback loop maintains an equilibrium between the rates of secretion of pituitary and ovarian hormones. The LH pulses stimulate the release of steroids from the ovary which act on the hypothalamus to reduce the frequency of GnRH pulses, and on the pituitary gland to reduce the response to GnRH. FSH stimulates the secretion of inhibin, which acts on only at the pituitary gland along with steroids to reduce the FSH response to GnRH. (Adapted from King, 1993).

II. Ovarian follicular dynamics in cattle

2.2. Population of follicles

Follicular populations can be divided into growing and non-growing pools of follicles (Kanitz, 2002). Primordial follicles, which are present throughout the reproductive life of the cow, are part of the non-growing pool, whereas the primary, secondary and tertiary follicles are part of the growing pool. Before the oocyte increases in diameter, it is enclosed by a single layer of squamous pre-granulosa cells (primordial follicle). The oocyte then increases in size and the squamous cells transform into cuboidal cells around the growing oocyte forming the primary follicle (Fortune et al., 2000). Non-cortical portions of the ovary may regulate the transition from primordial to primary follicles (Fortune et al., 1999). Recruitment of primordial follicles into the growing pool involves formation of layers of theca cells around these follicles and close interaction between theca cells and granulosa cells (Fortune et al., 1999).

2.3. Follicular waves

The first report on follicular dynamics was that of Hammond (1927), who collected ovaries from slaughtered cattle. This author noted that in the presence of a CL, the largest follicle on the ovary increases in size, and regresses with the regression of the CL. Rajakoski (1960) theorized that the growth of follicles in cattle occurs in a wave-like manner, and that cattle had two waves of follicular activity. The first wave starts around days 3 and 4 of the estrous cycle and results in non-ovulating follicle, and the second wave starts between days 12 and 14 of the estrous cycle and leads to an ovulatory follicle. The first-wave non-ovulatory follicle persists until day 12 of the estrous cycle, before

undergoing atresia, resulting in the emergence of the second follicular wave (Bane and Rajakoski, 1961). Pierson and Ginther (1984) used transrectal real-time ultrasonography to repeatedly study and monitor follicle (3 mm in diameter and more) development, and later (Pierson and Ginther, 1987) were able to support the two-wave hypothesis by Rajakoski (1960), after different sizes of follicles were monitored during the estrous cycle.

Transrectal ultrasonography has provided an efficient tool for studying follicular development as well as CL function in cattle, and it can be achieved without interfering with physiological processes such as the estrous cycle or pregnancy (Fortune, 1993). Sketches and graphics of individual follicles and CL's, by using ultrasonic examination studies have revealed more answers about antral follicular development in cattle and substantiated the occurrence of follicular waves (Rajamahendran and Walton, 1988; Sirois and Fortune, 1988). An antral follicular wave can be defined as a cohort of at least 3-6 antral follicles (≥ 3 or 4 mm in diameter) synchronized for their growth, followed by a selection of a DF from this cohort for continued growth and ovulation (Ginther et al., 1989b; Kulick et al., 1999). Follicular waves have been observed during the prepubertal period (Melvin et al., 1999), in pregnant cattle (Taylor and Rajamahendran, 1991), in postpartum periods (Murphy et al., 1990) and during the estrous cycle (Roche et al., 1999). The majority of cattle either have two or three follicular waves during the estrous cycle (Figure 2.3), with the DF being the ovulatory follicle at the time of luteal regression (Sirois and Fortune, 1988; Ginther et al., 1989b). In the three follicular waves cycle, the waves begin around days 2, 9, and 16; whereas in the two follicular wave cycle, the

waves start around days 2 and 11 (Sirois and Fortune, 1988). The length of the luteal phase during the estrous cycle, together with time of emergence of the second wave, appears to influence the number of follicular waves (Taylor and Rajamahendran, 1991; Kastelic and Ginther, 1991). A three-wave cycle tends to have a longer luteal phase than two-wave estrous cycle (Ginther et al., 1989b; Fortune, 1993). Animals with a two-wave cycle have significantly shorter luteal phases and subsequently shorter interovulatory intervals (Ginther et al., 1989b). If luteal regression occurs during the growth phase of the DF, final maturation and ovulation occurs. If however, luteolysis does not occur during the growth phase of the DF, atresia occurs to the DF. The second wave in a three-wave cycle emerges earlier than that of a two-wave cycle. The emergence of a wave can take place at the time of ovulation, when a future DF is detected from a synchronous cohort of follicles of minimum diameter of 3 mm (Kulick et al., 1999). A Few days after wave emergence, a selected follicle from the cohort becomes superior to other follicles (subordinate) and becomes dominant.

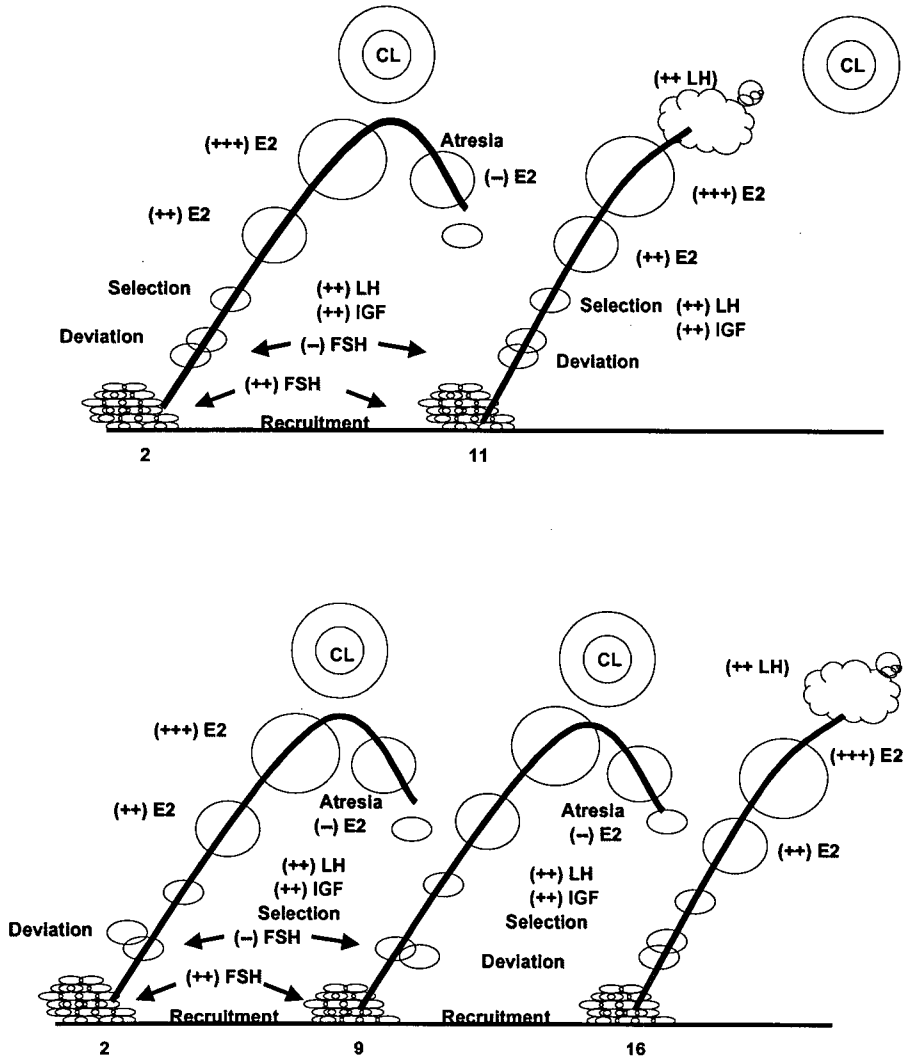

Figure 2.3. Schematic models of bovine ovarian follicular wave dynamics. The models explain the physiological terms, events and hormones associated with each follicular wave and its development during the estrous cycle. It is well established that the majority of bovine cycles consist of two or three follicular waves. In a two follicular wave cycle **(top graph)**, the waves start around days 2 and 11 of the estrous cycle, whereas, in a three follicular wave cycle **(bottom graph)**, the waves start around days 2, 9, and 16 of the estrous cycle. Each follicular wave consists of: a) emergence of a cohort (group) of similar sized growing follicles in a process called recruitment under the influence of FSH, and b) selection of the dominant follicle and the regression of the subordinate follicles. The selection process witnesses increases in LH, LH receptors, IGF and estradiol-17β in the dominant follicle, when compared to subordinate follicles. The first (two-wave cycle) and the first and second (three-wave cycle) wave dominant follicle are atretic because of the presence of functional CL and high progesterone concentrations. Following the regression of the CL, the ovulatory follicle ovulates. (Adapted from Ireland et al., 2000).

2.4. Recruitment

Recruitment defines the transition period when primordial follicles leave the non-growing phase and enter into the growing pool of the follicular population. In cattle, a follicle becomes antral when it reaches 0.2-0.4 mm in diameter, and continues to grow to a size of 3-5 mm in a period of at least 30 days (Lussier et al., 1987). During this period, the growth of the antral follicle is not dependent on the support of gonadotropins (Mihm et al., 2002). Subsequently, the growth of the follicle occurs in a wave-like manner and is dependent on FSH (Adams et al., 1992b). Following the emergence of the follicular wave, FSH accelerates the growth of the follicle beyond 4 mm in diameter, before it declines after one follicle is selected for dominance 2-3 days later and the remainder of the wave cohort undergo atresia (Mihm and Austin, 2002). During the estrous cycle, before puberty and postpartum, the start of every follicular wave is associated with a transient increase of FSH (Adams et al., 1992b; Kanitz, 2002).

2.5. Selection and characteristics of a dominant follicle

After recruitment, few follicles from the cohort continue growing while the remaining follicles undergo atresia. Unlike recruitment, the selection process coincides with declining concentrations of FSH that last for 2 to 3 days (Evans et al., 1997). All follicles greater than 5 mm have the capacity to suppress FSH during early stages of selection (Gibbons et al., 1997). During early luteal phase, inhibin has been shown to be the primary suppressant of FSH (Ginther et al., 2001).

Dominance can be defined as: (1) the day when the subordinate follicles (SF)

cease growth in diameter and become static/regressing, (2) the day when the DF reaches 8.5 mm in diameter, and (3) the difference between the DF and SF is not less than 2 mm in diameter (Mihm et al., 1994). A DF can also be identified with its increasing synthesis of E_2 and acquisition of LH receptors on the granulosa cells at the time of selection (8 to 9 mm in diameter) (Xu et al., 1995; Beg et al., 2001). A DF is recruited and selected during a follicular wave. An active DF is capable of preventing the growth of other follicles as well as the development of a new follicular wave (Rajamahendran et al., 1994). Each of the growing or static phases of the DF during the estrous cycle has a life span of 5 to 6 days (Rajamahendran et al., 1994). The future DF tends to have an advantage in terms of diameter size over the second largest follicle (SF). The greatest difference between the two largest follicles is called deviation. Based on 8 hour examination, deviation may occur within 8 h rather than 24 h (Ginther et al., 1997; Kulick et al., 1999). The difference in E_2 concentration between the DF and the SF occurs at the 8.5-8.9 mm range in diameter, with the increase in difference between them in LH receptor mRNA expression occurring at 8.0-8.4 mm range (Beg et al., 2001). The increased expression of LH receptor mRNA occurred one diameter range before an increased difference between the DF and the SF for diameter or E_2 concentrations (Beg et al., 2001). The same authors reported a progressive increase in free insulin-like growth factor (IGF)-1 and progressive decrease in IGF binding protein (BP)-2 in DF, relative to the SF. The future DF is detected about 6.9 h earlier than the future largest SF, with the DF having a diameter of 4 mm (Ginther et al., 1997; Kulick et al., 1999). The mean day of the beginning the process of deviation between the two largest follicles within a follicular wave is day 2.8, when the future DF possesses a mean diameter of 8.5 mm and

the SF mean is 7.2 mm in diameter (Ginther et al., 1996).

There are two types of follicle dominance, morphological and functional dominance. Functional dominance is defined as the ability of the DF to suppress the growth of SF and its ability to ovulate with the appropriate hormone treatment at an appropriate stage of follicle development (Fortune et al., 1991). Fortune et al. (1991) have shown that functional dominance was more important than morphological dominance (although it persists much longer) when determining the appropriate time of synchronization or superovulatory treatment. These authors also determined that, the maximum diameter of the DF of the first wave is no different from that of the second wave, regardless of the number of waves a cycle might have. Rajamahendran and Taylor (1991) have found that a single norgestomet ear implant (Synchromate-B) during the early or mid-luteal phase did not have any effect on the DF present. When, however, the implant was inserted during the follicular phase, in the absence of the CL, the DF was maintained for the duration of the treatment (9 days) and that the DF ovulated after the removal of the progestagen implant. Low levels of P_4 may result in persistence of the DF (Sirois sand Fortune, 1990; Savio et al., 1993). Increasing the plasma concentrations of P_4 leads to the demise of the DF and is associated with a decrease in LH pulse frequency (Taylor and Rajamahendran, 1994).

III. The corpus luteum

2.6. History

The CL was first diagramed by Regnier de Graaf in 1672 (Smith et al., 1994). He described the CL as a transient organ responsible for the survival of the number of embryos attained following fertilization. In 1897, John Beard stated that the CL suppresses ovulation during gestation before undergoing regression prior to parturition (Smith et al., 1994). In 1898, Prenant proposed that the CL is an endocrine gland, because it secretes one or more than one product into the blood circulation (Short, 1977). Frankel found that pregnancy was terminated following the removal of rabbit's ovaries which lead to Gustav Born hypothesizing that the CL is needed for implantation (Smith et al., 1994). In 1934, several research projects were conducted on the purification of P_4, the main hormone produced by the CL, which concluded that is needed for the establishment and maintenance of pregnancy (Smith et al., 1994; Niswender et al., 2000).

The CL is also known as the yellow body due to the high levels of the antioxidant β carotene which is a precursor of vitamin A (Graves-Hoagland et al., 1989). Young et al. (1995) suggested that antioxidants play a role in the protection of steroids of the ovary or other steroid producing tissues such as the adrenals from damage. They attributed this to the presence of the cytochrome P_{450} enzyme involved in the steroid synthesis, which secretes oxygen. Thus, infertility and reduced steroid production can be the result of antioxidant deficiencies (Luck et al., 1995).

2.7. Luteinization, establishment and function of the CL

Luteinization is defined as reorganization of follicular cells following the preovulatory LH surge, prior to ovulation. The follicular cells undergo morphological, endocrinological, and biochemical changes that are related to luteinization (Lipner, 1988; Niswender and Nett, 1988). Among the changes are: 1) the breakdown of the basement membrane which separate the theca from the granulosa cells, and 2) invasion of blood vessels into the ruptured wall of the antral follicular space, resulting in the development of extensive vascular network (Niswender et al., 1994). Blood flow increases with the increase in the CL weight (Damber et al., 1987). Luteinization is a transition period from a structure (preovulatory follicle), which mainly secretes E_2, to a structure (CL) which mainly secretes P_4. According to O'Shea et al. (1980), theca cells undergo hyperplasia, hypertrophy, and migration during the establishment of the CL.

Progesterone concentration defines the main function of the CL (Armstrong and Black, 1966). In cattle, adequate CL function, both structurally (maintenance of CL weight) and functionally (maintenance of P_4 secretion) is important for maintenance of pregnancy and prevention of luteolysis (Niswender et al., 2000). Processes such as the length of the estrous cycle, ovulation, and the fate of the embryo, may all depend on the function of the CL (Milvae et al., 1996). The bovine CL increases in size until mid-luteal stage, at which time it plateaus before decreasing in diameter during late diestrus stage (Mares et al., 1962). Concentrations of P_4 begin to increase days 3 to 12 of the estrous cycle and then stabilize until day 16 after which they rapidly decrease following regression of the CL (Hansel and Echternkamp, 1972; Rajamahendran et al., 1976).

Inadequate CL function, also called luteal dysfunction, is characterized by an estrous cycle of normal duration and low peripheral P_4 (Lamming et al., 1981). Development of the conceptus is related to concentrations of P_4 and ability of the conceptus to secrete INF-t (Thatcher et al., 2001). This fact was supported by the work of Henricks et al. (1971) and Lamming et al. (1989) who both reported that cows with low P_4 concentrations between days 10 and 16 post breeding did not maintain pregnancy.

2.8. Progesterone synthesis

The steroidogenesis process that results in the production of P_4 is illustrated in Figure 2.4. Cholesterol is the main source and precursor for the production of the luteal P_4. Cholesterol can be obtained from low density lipoproteins (LDL) or high density lipoproteins (HDL) found in the blood (Anderson and Dietschy, 1978; Azhar and Meanon, 1981), acetate (Hinshelwood et al., 1993), lipid droplets in luteal cells (Hansel et al., 1987), or it may exist in unesterified form in small and large luteal cells (Christie et al., 1979). However, the production of cholesterol is mainly from lipoproteins in blood (Anderson and Dietschy, 1978; Azhar and Meanon, 1981). Studies using sheep and cattle have shown that either LDL or HDL stimulates an increase in P_4 production in luteal cells (Wiltbank et al., 1990; Carroll et al., 1992). Lipoproteins (either LDL or HDL) bind to their luteal plasma membrane-specific receptors, stimulating the release of cholesterol. Cholesterol binds to a sterol carrier protein (SCP) (Gibori, 1993) and is transported initially from the cytoplasm to the mitochondria (Rodgers et al., 1986). Upon arrival into the mitochondria it moves from the outer to the inner mitochondrial membrane, which is the site of the rate limiting side chain cleavage enzyme activity, the cytochrome P_{450} side

chain cleavage enzyme (Niswender et al., 2000). This enzyme converts cholesterol to pregnenolone, a C-21 compound (Milvae et al., 1996). Pregnenolone then diffuses into the endoplasmic reticulum from the mitochondria and catalyzes to progesterone (Milvae et al., 1996). The enzyme responsible for this catalytic step is Δ^5 3β-hydroxy steroid dehydrogenase, Δ^5 Δ^4, isomerase (3-βHSD) (Labrie et al., 1992), which is distributed throughout the bovine luteal cells (Conley et al., 1995).

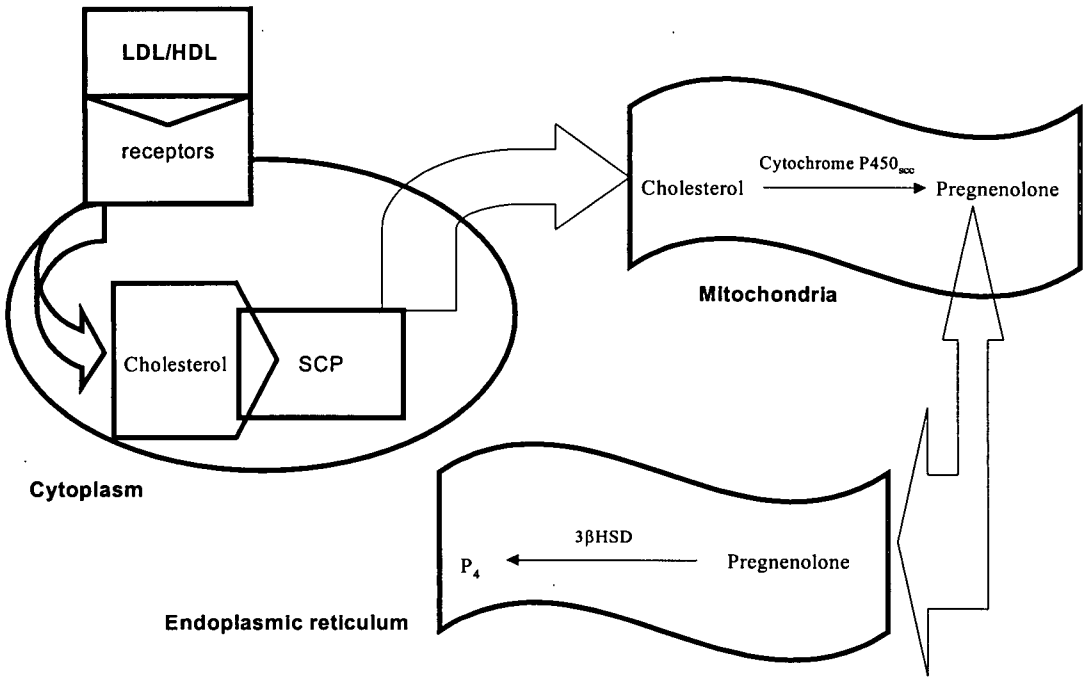

Figure 2.4. Schematic diagram for progesterone biosynthesis in a luteal cell. Cholesterol is the main source and precursor for the production of the luteal progesterone. The production of cholesterol is mainly from lipoproteins (LDL or LDL) in blood. Free cholesterol binds to a sterol carrier protein (SCP) and is transported initially from the cytoplasm to the mitochondria. Cholesterol is then transported from outer to inner mitochondrial membrane. Cholesterol is converted to pregnenolone by the cytochrome P_{450} side chain cleavage enzyme, transported out of mitochondria, and converted to progesterone by Δ^5 3β-hydroxy steroid dehydrogenase, Δ^5 Δ^4, isomerase (3-βHSD), which is present in the smooth endoplasmic reticulum. (Adapted from Niswender et al., 2000).

2.9. Corpus luteum heterogeneous cells

Cell types identified thus far in the CL's are: small luteal cells (SLC), large luteal cells (LLC), endothelial cells, and fibroblasts (Hansel et al., 1991). In sheep and cattle the SLC and LLC are identified as steroidogenic active cells (Urseley and Leymarie, 1979; Fitz et al., 1982; Hansel et al., 1987). According to O'Shea et al. (1989), 60-70% of luteal cells are non-steroidogenic. The SLC are derived from the theca interna of the follicle, and range from 10-20 μm in diameter (Alila and Hansel, 1984). The SLC represent about 20% of the total CL volume and approximately 25% of the CL total cell number. The LLC are at least 25 μm in diameter and are derived from either the granulosa or theca cells of the follicle, depending on the stage of the estrous cycle (Hansel et al., 1987). They comprise 40% of the CL total volume, compared to only 10% of the total CL cell number they constitute (Hansel et al., 1987). While luteinized granulosa cells in culture showed higher concentrations of cytochrome P_{450scc} enzyme, with greater basal P_4 synthesis than luteinized theca cells, luteinized theca cells in culture showed more response to LH, cAMP, and forskolin on the basis of progesterone production (Meidan et al., 1990). Large luteal cells produce most of the basal P_4 in vitro (Urseley and Leymarie, 1979; Hansel et al., 1991), and approximately 80% of the in vivo secreted P_4 in ovine (Hansel et al., 1991). While SLC are more responsive to the stimulating effects of LH, the LLC are more responsive to $PGF_{2\alpha}$ (Urseley and Leymarie, 1979; Alila et al., 1988). Small luteal cells consist of eccentric cup-shaped nucleus, tubular cristae mitochondria, smooth and rough endoplasmic reticulum, and smooth surface membrane which include microvilli. The mitochondria are located near the nucleus. The LLC include more mitochondria, a round nucleus that is located in the center, and endoplasmic reticulum

that is well-developed (Koos and Hansel, 1981). Large luteal cells contain secretory granules, which may be essential for storing and transporting material for exocytosis. These granules are believed to secrete peptides such as oxytocin, relaxin and neurophysin (Fields et al., 1980).

2.10. Mechanisms for controlling CL function

There are two mechanisms involved in the control of the CL function; the first is the luteotropic mechanism responsible for the induction of the CL function; and the second mechanism is the luteolytic mechanism involved in the inhibition of the CL function (Niswender et al., 2000).

2.10.1. LH and the CL (luteotropic mechanism)

After determining that human chorionic gonadotropin (hCG) overturned the inhibition by oxytocin on the CL weight and P_4, LH was proposed as the major luteotropin in cattle (Simmons and Hansel, 1984). This observation was later supported by Donaldson and co-workers, when the work was conducted using LH (Simmons and Hansel, 1984).

2.10.1.1. Profile of LH during the luteal phase of the estrous cycle

Luteinizing hormone is secreted in pulses, with variable amplitude and frequency (Rahe et al., 1980). According to these scientists, in early luteal phase, the pulses are of low amplitude and high frequency; whereas, in the mid-luteal phase, the pulses are of high amplitude and low frequency. During luteolysis, LH tends to have high amplitude

and high frequency. Rahe et al. (1980) also found that LH concentrations in the cow were similar during the early and mid-luteal periods. Luteinizing hormone is regulated through negative feedback (Kazama and Hansel, 1970), and positive feedback (Hobson and Hansel, 1972) mechanisms, by P_4 and E_2, respectively. Sheep treated with GnRH antagonist between days 4 and 11 did not show any effect on the P_4 levels (McNeilly et al., 1992). This has been attributed to GnRH antagonist, which abolishes the LH pulsatile secretion, indicating that low levels of basal LH may be sufficient to maintain adequate CL function. However, treatment with GnRH antagonist in cattle before day 12 of the estrous cycle reduced P_4 concentration, whereas GnRH antagonist treatment between days 12 and 17 did not (Peters et al., 1993). Hunter (1991) stated that episodic release of LH prior to ovulation might be of significance for inducing maturation of the DF. Pulsatile LH is also necessary for the secretion of P_4 by the luteal cells. In cattle, the frequency of episodic LH during the follicular phase (7 ~ pulses every 12 h) is significantly different from the frequency during the luteal phase (3 ~ pulses every 12 h) of the estrous cycle (Cupp et al., 1995). Episodic release of LH is more significant for CL development rather than for CL function (Peters et al., 1994). A study by Quintal-Franco et al. (1999) found that episodic release of LH 48 h prior to initiation of preovulatory LH surge is important for the development of cattle CL. Cows treated with GnRH antagonist 48 h before the preovulatory LH surge had smaller CL size, compared to control cows. Studies involving women showed that abnormal release (either high or low) of episodic LH during the follicular phase of the menstrual cycle resulted in failed fertility (Souls et al., 1989; Schweiger et al., 1989).

2.10.1.2. Action of LH

Luteinizing hormone luteotropic action is accomplished by binding to its plasma membrane rhodopsin/β_2-adrenergic receptor subfamily of G protein-coupled receptors (GPCRs) (Ascoli et al., 2002; Figure 2.5). When LH binds to its receptor on the surface of the luteal cells, it causes a change in the G-protein (signal transducers) affinity for Mg^{+2} (Niswender et al., 2000). Prior to LH-receptor binding, the G-protein subunits (α,β, γ) are attached together, with guanosine diphosphate (GDP) bound to Gα protein. At the time of binding of LH to its receptor, GDP is converted to guanosine triphosphate (GTP) on the G_α protein, resulting in an increase in Mg^{+2} affinity. Then, G_α-GTP (the active form) may separate from the G$\beta\gamma$ subunits and enter the cytoplasm, where it stimulates the catalytic activity of adenylate cyclase (cellular system), resulting in the production of cAMP from ATP (Niswender et al., 2000). Acting as a second messenger signal, cAMP activates protein kinase A (PKA) by binding to the regulatory subunits. Protein kinase A has been found to stimulate the release of cholesterol for transport to the mitochondria, the site of side chain cleavage, and the rate limiting step in the production of P_4 (Wiltbank et al., 1993; Niswender et al., 1994).

2.10.1.3. Concentration of LH receptors

Luteinizing hormone receptor (both density and mRNA) is highest during the mid-luteal phase and lowest during early and late luteal phase of the estrous cycle (Garverick et al., 1986; Smith et al., 1996). However, only 2% of the total receptors are occupied receptors (Garverick et al., 1986). These authors found that P_4 levels (high) during the mid-luteal phase were highly correlated with the occupied receptors. In

addition, only the occupied receptors were able to activate G-protein, adenylate cyclase enzyme, and cAMP second messenger system (Stryer, 1988).

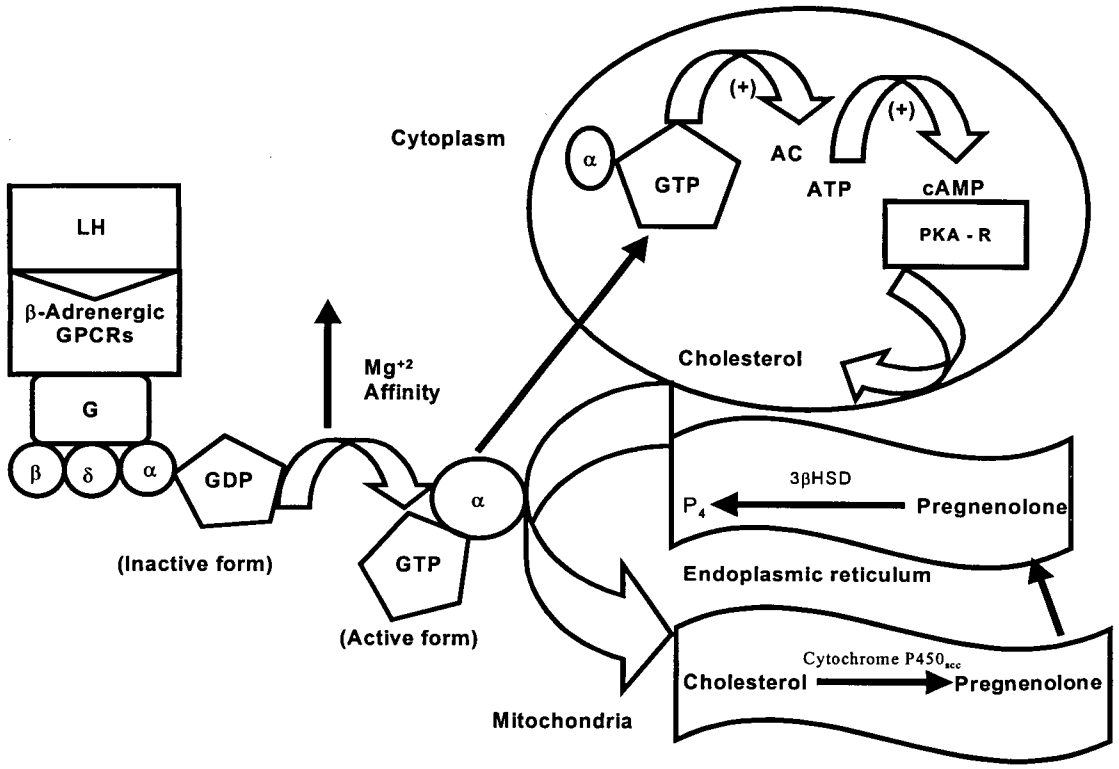

Figure 2.5. Schematic representation of LH luteotropic action in the luteal cell. LH binds its plasma membrane rhodopsin/β_2-adrenergic receptor subfamily of G protein-coupled receptors (GPCRs) in the luteal cells and causes a change in the G-protein (signal transducers) affinity for Mg^{+2}. Prior to LH-receptor binding, the G-protein subunits (α,β, γ) are attached together, with guanosine diphosphate (GDP) bound to Gα protein. At the time of binding of LH to its receptor, GDP is converted to guanosine triphosphate (GTP) on the G_α protein, resulting in an increase in Mg^{+2} affinity. Then, G_α-GTP (the active form) separates from the G$\beta\gamma$ subunits and enter the cytoplasm, where it stimulates the catalytic activity of adenylate cyclase (cellular system), resulting in the production of cAMP from ATP. Being as a second messenger signal, cAMP activates protein kinase A by binding to the regulatory subunits. Protein kinase A has been found to stimulate the release of cholesterol for transport to mitochondria, the site of side chain cleavage, the rate limiting step in the production of P_4. Pregnenolone is converted to P_4 in the smooth endoplasmic reticulum. (Adapted from Niswender et al., 2000).

2.10.2. $PGF_{2\alpha}$ and the CL (luteolytic mechanism)

2.10.2.1. Luteolysis

Progesterone is essential throughout pregnancy to maintain the uterus in a secretory and quiescent state, which is essential to nurture and maintain the conceptus until it is fully developed. In cattle, the uterus regulates life span of the CL by secreting a $PGF_{2\alpha}$. This substance serves as a "maternal recognition signal" in non-pregnant, cycling animals (King, 1993). Luteolysis refers to the normal demise of the CL in non-pregnant animals and the initiation of new reproductive cycle. Prostaglandin $F_{2\alpha}$ causes premature luteal regression, following direct infusion into CL of monkeys (Auletta et al., 1984). Several processes may occur at the onset of luteal regression that may include: decrease in P_4 concentrations in serum (Diekman et al., 1978), decrease in luteal weight (Diekman et al., 1978), accumulation of lipid droplets in the cytoplasm of luteal cells, degeneration of capillaries, increase in lysosome numbers (Nett et al., 1976), and decline in the number of steroidogenic luteal cells (Braden et al., 1988).

There are several proposed mechanisms as to how $PGF_{2\alpha}$ exerts its action and causes luteal regression. During the luteal phase, large follicles secrete increasing amounts of E_2, which enhances the induction of receptors for both E_2 and oxytocin (Silvia et al., 1991). Silvia et al. (1991) proposed (Figure 2.6) that pulsatile oxytocin from the posterior pituitary binds its receptors located in the endometrium, stimulating the release of tonic $PGF_{2\alpha}$. Tonic $PGF_{2\alpha}$ triggers the release of episodic oxytocin from the CL, which then binds the oxytocin receptors in the endometrium, releasing episodic $PGF_{2\alpha}$ from the uterus. The uterine $PGF_{2\alpha}$ initiates a positive feedback loop involving

luteal oxytocin and uterine $PGF_{2\alpha}$ (Silvia et al., 1991). According to these authors, luteolysis occurs through the counter-current mechanism, in which $PGF_{2\alpha}$ is transferred between the uterine vein and ovarian artery.

Tsai and Wiltbank (1997) have suggested that initial $PGF_{2\alpha}$ released in response of oxytocin-stimulated receptors causes the LLC to produce considerable amounts of local $PGF_{2\alpha}$. The mechanism involves uterine $PGF_{2\alpha}$ stimulating phospholipases to liberate arachidonic acid (after minutes of increased free intracellular calcium), and increased cyclooxygenase (prostaglandin G/H synthase-2 [PGHS-2] mRNA and protein. This enzyme converts arachidonic acid to PGH_2. Under normal physiological conditions, PGHS-2 is found in minute amounts (O'Neill and Ford, 1993).

In a study about the transport of cholesterol across the mitochondrial membrane, Clark et al. (1994) isolated and sequenced a mitochondrial protein which stimulated steroidogenesis in transfected MA-10 Leydig tumor cells. These scientists named this protein Steroidogenic Acute Regulatory protein (StAR; 37-kDa) and proposed that it facilitates the transport of cholesterol from the mitochondrial membrane to provide substrate for the synthesis of P_4 by the P-450scc enzyme (Stocco and Clark, 1996). Researchers have found that $PGF_{2\alpha}$ reduced the levels of StAR mRNA in ovine and bovine luteal tissue (Juengel et al., 1995; Pescador et al., 1996). This suggests one mechanism of antisteroidogenic action by $PGF_{2\alpha}$. It was reported that hypophysectomy and the removal of LH caused a decline in the StAR mRNA in luteal tissue, and restoration of LH was accompanied by the restoration of StAR mRNA (Juengel et al.,

1995). Since $PGF_{2\alpha}$ reduces LH receptors, it may act at the cellular level and diminish StAR (Juengel et al., 1995).

It is also hypothesized that the antisteroidogenic action of $PGF_{2\alpha}$ during luteolysis, includes the role of endothelin-1 (ET-1), a 21-amino acid peptide synthesized by endothelial cells. It has been suggested that ET-1 inhibits gonadotropin-stimulated P_4 production by porcine granulosa cells (Flores et al., 1992; Iwai et al., 1991) and bovine luteal tissue (Girsh et al., 1995; Girsh et al., 1996a,b). Endothelin-1 inhibited P_4 production from steroidogenic luteal cells, an effect that was reversed by an ET-1 receptor antagonist (Girsh et al., 1996a,b). Also, it has been shown that $PGF_{2\alpha}$ simulated an increase in the ET-1 levels in luteal tissue, and ET-1 receptor antagonist blocked $PGF_{2\alpha}$ inhibition of P_4 production by luteal tissue (Girsh et al., 1996a,b).

2.10.2.2. Precursors of prostaglandins

Prostaglandins are part of compounds called eicosanoids because they contain 20 carbon atoms. Eicosanoids also include prostacyclins, thromboxanes and leukotrienes. Prostaglandin $F_{2\alpha}$ is derived from a common precursor polysaturated fatty acids called arachidonate $(CH_3(CH_2)_4(CH{=}CHCH_2)_4(CH_2)_2COO^-$. Arachidonate are derived from linoleate $(CH_3(CH_2)_4(CH{=}CHCH_2)_2(CH2)_6COO^-$. The information in this paragraph is taken from Stryer (1988).

2.10.2.3. Origin of luteolytic prostaglandin $F_{2\alpha}$

Uterine prostaglandin $F_{2\alpha}$ is the main factor responsible for luteal regression and

cessation of P_4 production in domestic farm animals and most rodents (Knickerbocker et al., 1988). Loeb (1923, 1927) was the first to report the importance of the uterus in the control of CL (A review by McCracken et al., 1999). In these reports, it was shown that cyclic guinea pigs subjected to hysterectomy exhibited abolished cycles and an abnormal persistence of the CL's. Similar effects were observed in cows, mares, pigs, and sheep (Anderson et al., 1969). Goding et al. (1972) found $PGF_{2\alpha}$ to have luteolytic effects in sheep. Ginther (1974) indicated that the effects of $PGF_{2\alpha}$ were localized, since removal of the uterine horn ipsilateral to the CL prevented luteolysis, whereas removal of the uterine horn contralateral to the CL had no effect on the CL life span.

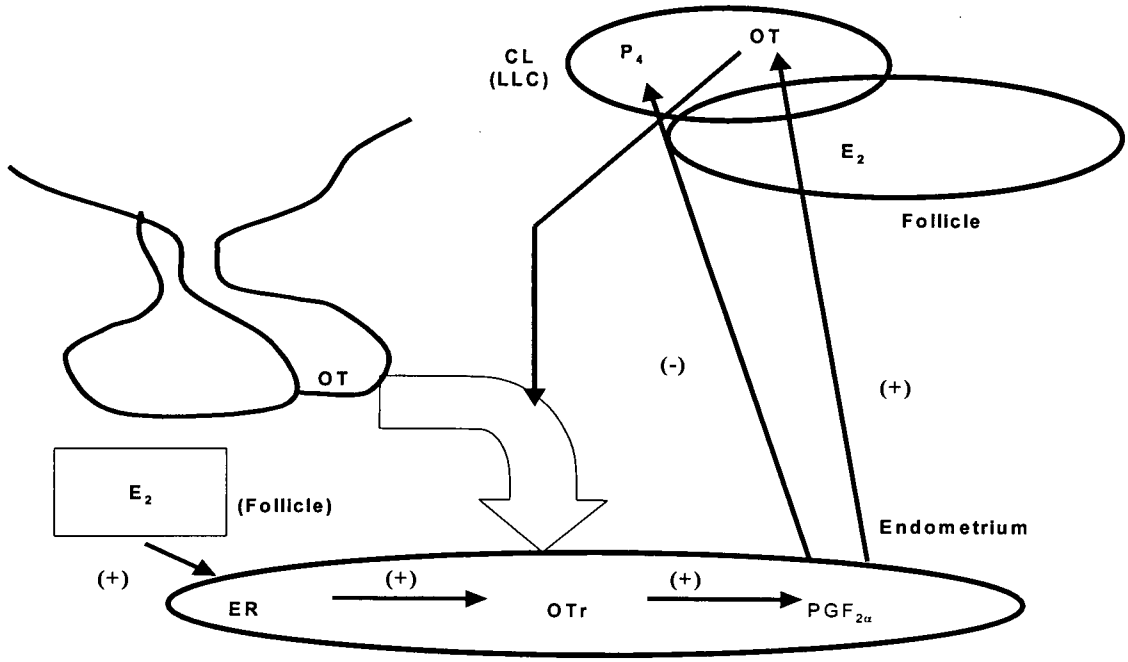

Figure 2.6. A model of luteolysis as proposed by Silvia et al. (1991). Corpus luteum regression is a uterine-dependent event in cattle involving the episodic secretion of $PGF_{2\alpha}$ from the endometrium. Episodic $PGF_{2\alpha}$ release from the uterine endometrium requires secretion of oxytocin from the CL and neurohypophysis. The secretion of oxytocin and up-regulation of uterine oxytocin receptors may be as response to E_2 concentrations during mid-luteal phase. Oxytocin in turn binds to endometrial oxytocin receptors to stimulate $PGF_{2\alpha}$ release during late diestrus. The pulsatile nature of endometrial $PGF_{2\alpha}$ secretion is critical for rapid and effective occurrence of CL regression. Luteolysis occurs through the counter-current mechanism, in which $PGF_{2\alpha}$ is transferred between the uterine vein and ovarian artery.

2.10.2.4. Action of $PGF_{2\alpha}$

Prostaglandin $F_{2\alpha}$ exerts its antiluteolytic actions by binding to its G-protein receptors (Sakamato et al., 1994; Figure 2.7) mainly in the large luteal cells (Sakamato et al., 1995) through a second messenger system of protein kinase C (PKC). Prostaglandin $F_{2\alpha}$ activates phospholipase C (Davis et al., 1987) and hydrolyzes membrane phosphatidyl inositol 4,5-bisphosphate (PIP_2) to inositol-1, 4, 5-triphosphate (IP_3) and 1,2-diacylglycerol (DAG) (Berridge, 1987). Diacylglycerol causes an increase in PKC affinity for calcium and IP_3 liberates calcium from endoplasmic reticulum, resulting in an increase in free intracellular calcium (Nishizuka, 1986) and activation of PKC. An increase in intracellular calcium appears to be the luteolytic determinant factor, as evidenced in ovine large luteal cells when $PGF_{2\alpha}$ increased free intracellular calcium concentrations (Wiltbank et al., 1991).

Treatment with $PGF_{2\alpha}$ decreased LH receptors in ewes (Diekman et al., 1978). The second messenger system of free intracellular calcium may be responsible for the inhibitory action of $PGF_{2\alpha}$. Calcium may act to hydrolyze cAMP to the inactive AMP and terminate cAMP pathway (LH-LH receptor pathway) through action of the phosphodiestrase enzyme that is regulated by calcium (Berridge, 1993). High levels of arachidonic acid or lypooxygenase products, result in extreme levels of intracellular calcium in large luteal cells, which initiates luteolysis, either by producing oxytocin from secretory granules in a way of exocytosis, or by calcium activated DNA fragmentation (apoptosis) leading to programmed cell death (Hansel et al., 1991).

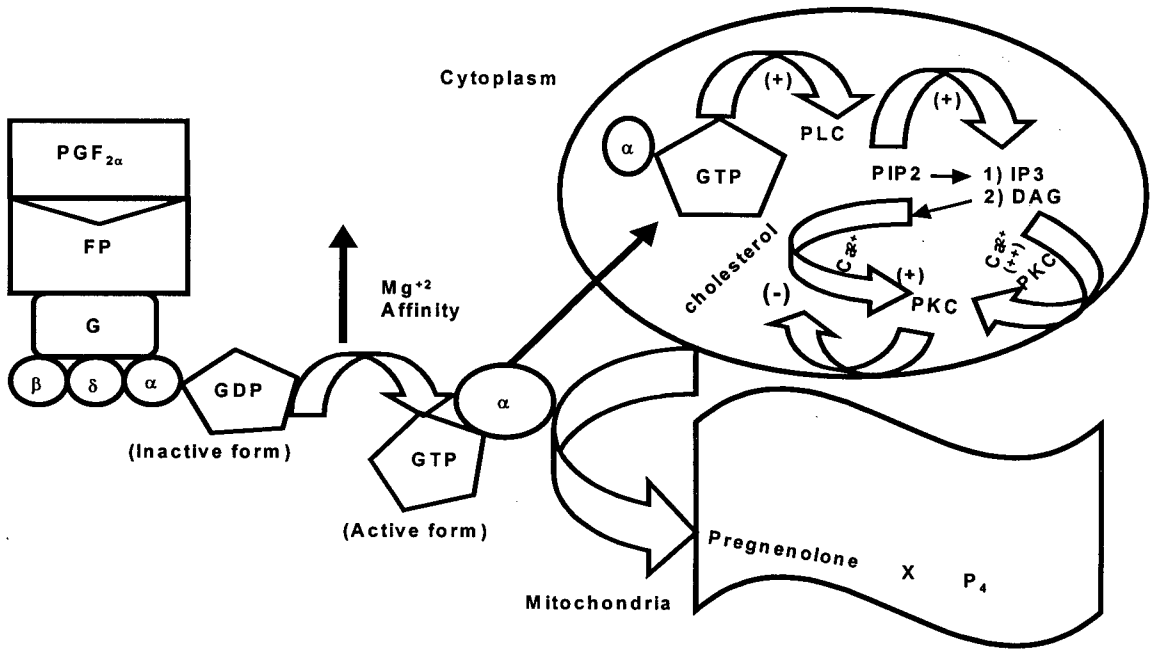

Figure 2.7. Schematic model of luteolytic action of $PGF_{2\alpha}$ in the luteal cell. Prostaglandin $F_{2\alpha}$ exerts its luteolytic actions by binding to its plasma membrane receptors (FP receptors), G-protein receptors mainly in the large luteal cells through a second messenger system of protein kinase C (PKC). Prostaglandin $F_{2\alpha}$ activates phospholipase C and hydrolyzes membrane phosphatidyl inositol 4,5-bisphosphate (PIP_2) to inositol-1, 4, 5-triphosphate (IP_3) and 1,2-diacylglycerol (DAG). Diacylglycerol act to increase PKC affinity for calcium and IP_3 liberate calcium from endoplasmic reticulum, resulting in increase in free intracellular calcium and activation of PKC. PKC may in turn prevent the transport of cholesterol into the mitochondria and the conversion to pregnenolone, or it can prevent the diffusion of pregnenolone into the smooth endoplasmic reticulum and the conversion to progesterone. (Adapted from Niswender et al., 2000).

2.10.2.5. Concentration of $PGF_{2\alpha}$

Concentrations of $PGF_{2\alpha}$ increase in the uterine venous drainage, uterine tissue, and uterine secretions during periods of expected luteolysis (King, 1993). Administration of indomethacin ($PGF_{2\alpha}$ inhibitors) extends the life span of the CL. A close relationship exists between high concentrations of $PGF_{2\alpha}$ in the ovarian vein and increased amounts in the ovarian artery around time of luteolysis (King, 1993). Progesterone exposure is necessary in order for the uterus to produce $PGF_{2\alpha}$, (Knickerbocker et al., 1988).

2.10.2.6. Concentration of $PGF_{2\alpha}$ receptors

The $PGF_{2\alpha}$ FP receptors mRNA in ovine CL have been found to be highest on day 10 of the estrous cycle (Graves et al., 1995). These latter authors reported a reduction in FP receptors on day 16, and this reduction was associated with the falling levels of circulating P_4. Luteolysis triggered a reduction of FP receptors (Garves et al., 1995). Infusion of $PGF_{2\alpha}$ in vivo caused down-regulation of high affinity FP receptors in ovine CL (Lamsa et al., 1992). The greatest density for FP receptors occurred during day 10 of the luteal phase and lowest on day 15 in non-pregnant ewes (Wiepz et al., 1992). However, Wiltbank et al. (1995) found no significant difference between developing (days 2 and 4) and active (days 6 and 10) bovine CL in either affinity or concentration of FP receptors. This was in contrast to the results of Rao et al. (1979) who found FP receptor density progressively increased from day 3 and peaking on day 20, and the affinity for the FP receptor on day 13 was 203 times lower than that of day 20. Rueda et al. (1995) reported that the concentrations of FP receptors decreased with the falling concentrations of P_4. In NIH 3T3 cells there is evidence for two forms of the FP

receptors, one that couples to adenylate cyclase and does not exhibit selectivity between $PGF_{2\alpha}$ and PGE_2 and another which couples to phospholipase C and shows a 40-fold selectivity towards $PGF_{2\alpha}$ over prostaglandin-E_2 (PGE_2) (Gusovsky, 1991).

2.11. In vitro CL function

The ability of CL to produce P_4 when isolated from the animal and incubated in vitro has confirmed that the main function of the CL is the production of P_4 (Armstrong and Black, 1966). Corpus luteum enucleation has been a useful means to provide answers about the direct effects of stimulatory and inhibitory hormones/factors on synthesis of in vitro P_4 as well as the mechanisms of these hormones/factors within different cells of the CL (Hansel et al., 1991). Progesterone production per gram of luteal tissue did not differ until day 14 after estrus (Armstrong and Black, 1966). This was in agreement with the in vivo study conducted by Mares et al. (1962) in which they found that the CL increased in size until day 11 of the estrous cycle when then it became static before undergoing regression after day 15 of the estrous cycle. Luteinizing hormone was found to be the only significant luteotropin and it was established that its action was through the cAMP second messenger system (Hansel et al., 1991). The effect of LH in vitro varies with the stage of the luteal phase (Armstrong and Black, 1966). Luteinizing hormone increased P_4 production during early and especially mid-luteal phase (Armstrong and Black, 1966; Redmer et al., 1988). It has been suggested that during the early stages of the estrous cycle, LH is high and therefore CL may not respond to LH in vitro (Armstrong and Black, 1966). A mid-luteal phase CL with its production of high P_4 exerts a negative feedback on LH and therefore is more responsive to LH in vitro

(Armstrong and Black, 1966). In cattle, both small and large luteal cells have receptors for LH (Niswender et al., 2000). It was proposed that LH binds its receptors in small luteal cells and stimulates protein kinase activity and an increase in P_4 (Harrison et al., 1987; Hansel et al., 1991; Niswender et al., 2000).

The large luteal cells have been shown to possess receptors for prostaglandin-I_2, PGE_2 and $PGF_{2\alpha}$ (Niswender et al., 2000). Prostaglandins E_2 and I_2 have luteotropic properties, whereas $PGF_{2\alpha}$ is mainly luteolytic (Niswender et al., 2000). However, $PGF_{2\alpha}$ has also been shown to stimulate P_4 secretion in vitro above control levels when either CL slices or CL cells were incubated between 1 to 4 h (Speroff and Ramwell, 1970; Redmer et al., 1988; Hansel et al., 1991). Hansel et al. (1991) stated that the stimulatory effect occurs in SLC via phospholipase C mechanism. Hall and Robinson (1979) showed inhibitory effects of $PGF_{2\alpha}$ on rat luteal tissue with respect to P_4 synthesis. In LLC, arachidonic acid stimulated $PGF_{2\alpha}$, which inhibits P_4 secretion, but in small luteal cells, arachidonic acid stimulated production of prostaglandins I_2 and E_2 or stimulated the uptake of cholesterol and the increase in P_4 production (Del Vecchio et al., 1995a,b).

IV. Current status of estrus synchronization protocols in cattle

2.12. Progestins in synchronization

2.12.1. Subcutaneous injections

Progesterone was the hormone originally used to synchronize the bovine estrous cycle (Lamond, 1964; Gordon, 1976). It has been used to induce estrus synchronization in anestrual postpartum cows (Foot and Hunter, 1964; Cavestany et al., 2003).

Progesterone dissolved in oil was first used to exert a negative feedback on the pituitary by blocking gonadotropin secretion, estrus and ovulation (Christian and Casida, 1948). The procedure included an injection of P_4 solution (50 mg; Ulberg et al., 1951) daily, for up to 20 days. The time range of when cows returned to estrus varied considerably and fertility was very low as a result of the treatment (Trimberger and Hansel, 1955; Lamond, 1964). The P_4 treatment disabled spermatozoa ability to survive in the female reproductive tract, leading to the low fertility, as suggested by Lauderdale and Ericsson (1970).

2.12.2. Oral progestins

In 1960, medroxyprogesterone acetate (MAP) and chlormadionone progesterone acetate (CAP), synthetic progestins were used to suppress estrus and ovulation (Hansel and Malven, 1960). In 1962, melengesterol acetate (MGA; oral progestin) was developed to control both estrus and ovulation (Patterson et al., 1990). MGA is several hundreds times more potent than both MAP and CAP (Patterson et al., 1990). It was first tried for 10-18 days (long term) by giving 0.5-1.0 mg/head/day, in the feed, causing estrus to occur within 6 days after treatment in 70% of animals (Zimbelman et al., 1970). This rate was not significantly different from that of untreated animals. The conception rate (CR), however, was 30% lower than that of untreated animals. In another study, beef cows were fed MGA for 20 days with exogenous P_4 (20 mg/ml) given two days prior to the end of the MGA treatment (Anderson and Day, 1998). This treatment resulted in 100% estrus synchrony in cyclic cows, with 64% of cows were in estrus on days 4 and 5, and 36% on days 3, 6 and 7. This treatment yielded 85% CR, similar (87%) to that detected in cows

treated with MGA only. However, treatment with MGA late in the cycle resulted in delay of estrus and inhibition of ovulation (Beal et al., 1990). The researchers attribute their results to high E_2 concentrations 7 days before ovulation.

2.12.3. Progestins; silastic, sponges, and vaginal devices

The use of silastic progestin implants was first conducted by Dziuk and Cook (1966). The implants were filled with MGA and were inserted in the neck between 22 to 64 days (Dziuk et al., 1966). Cows were in estrus (64%) within 72 h following the removal of the implants, with fertility being not high. However, Curl et al. (1968) reported 68% CR and estrus synchrony within 48 h after synchronization with subcutaneous implants impregnated with northandrolone.

Another approach for estrus synchronization was the use of sponges, impregnated with oil containing progestagen and placed in the anterior part of the vagina for 18 to 21 days. The suppression of estrus was achieved with this method (Carrick and Shelton, 1967) and upon removal of the sponge, animals were in estrus within 72 h, but the fertility was lower than that of the control animals (Hansel and Schechter, 1972).

Later, the progesterone-releasing intravaginal device (PRID; Abbott Laboratories Ltd) was discovered and used as a progestagen placed in the anterior part of the vagina (Mauer et al., 1975). Increasing levels of LH were noticed when PRID devices were inserted into cows during the estrous cycle (Roberson et al., 1989). Another intravaginal P_4 device was controlled internal drug release (CIDR-B®; 1.9 g of progesterone), which

was developed jointly at the Ruakura Agricultural Research Center and the Agricultural Division of the Carter Holt Harvey Plastic Products Group Ltd., both of Hamilton, New Zealand (Macmillan and Peterson, 1993). Duration of CIDR treatment as well as the day of the estrous cycle when the CIDR device is inserted play important roles in determining fertility rates. Treating with CIDR late in the cycle and for 14 days caused the DF to become persistent (Stock and Fortune, 1993). The same study found that only one of six heifers (17%) that ovulated a persistent follicle after CIDR treatment conceived, compared to five of six (83%) of non-CIDR heifers.

Although prolonged exposure (i.e. 14-18 days) to low P_4 concentration results in higher estrus synchrony, it also results in persistence of the DF. Lower fertility rates has been attributed to the maintenance of the DF and ovulation of an aged oocyte (Beal et al., 1988; Savio et al., 1993). The persistence of a large, and DF is accompanied with an increase in pulsatile release of LH (Rajamahendran and Manikkam, 1994). However, 7-10 day progestagen treatments (short term) administered late in the estrous cycle resulted in lower fertility (Beal et al., 1988; Brink and Kiracofe, 1988). In addition, abnormal embryonic development and reduced fertility can be the result of prolonged exposure to E_2, both in cows (Breuel et al., 1993; Wehrman et al., 1993), and rats (Butcher and Pope, 1979). Six days prior to estrus, cows with persistent follicles had twice as much of E_2 in their plasma than cows lacking persistent follicles, which might have resulted in embryonic mortality (Ahmad et al., 1995). This was in agreement with Garcia-Winder et al. (1987), who found that cows treated for 9 days with norgestomet resulted in the largest follicle expressing its dominance over other follicles in the cohort, with the DF

having more E_2 concentrations. Johnson and Lewis (1993) related lower conception rates in sheep to elevated E_2 concentration. Further, abortion in women was partly related to high levels of E_2 in follicular fluid and in serum per follicle (Lewinthal et al., 1987).

Administering P_4 or its analogues late in the cycle can result in lower fertility. Furthermore, prolonged treatment of P_4 can also result in lower fertility. This has been mainly attributed to altered follicular growth, increased E_2 concentration as wells as increased LH pulse frequency prior to ovulation, spermatozoa inability to survive, which collectively could result in abnormal embryo development. Hence, to reduce the incidence of DF persistence, exogenous P_4 may be supplemented with traditional progestagen treatments. Adams et al. (1992a) and Rajamahendran and Manikkam (1994) observed that plasma P_4 levels greater than the physiological levels caused the DF to decrease in maximum diameter; whereas, P_4 levels less than normal physiological levels increased the diameter of the follicle and caused it to become persistent. After administering two PRID's, Roberson et al. (1989) found that LH pulse frequency remained low, even in the absence of the CL (Savio et al., 1993; Stock and Fortune, 1993).

2.13. Estrogens and progestins in synchronization

Estrogens when administered early in the estrous cycle caused the demise of the CL (Loy et al., 1960; Wiltbank, 1966). Accordingly, short-term (9 to 14 days) synchronization treatments including progestins and estrogens were developed. Synchromate B (SMB) is one of the synchronization methods which has been used in

cattle operations, particularly in beef cattle. This "short term" (9 days) treatment that consists of 6 mg norgestomet implant, 5 mg of estradiol valerate and 3 mg of norgestomet given at the time of implant insertion successfully synchronized estrus and induced estrus in non-cycling beef heifers (Wiltbank and Gonzalez-Padilla, 1975). Tight estrus synchrony (24 to 48 h) was reported following SMB treatment (Miksch et al., 1978; Spitzer et al., 1978). Timed-breeding-pregnancy rate of heifers due to SMB treatment was 51%, compared to that of heifers inseminated 12 h post natural estrus (Mares et al., 1977). However, treating beef cows with SMB late in the estrous cycle caused the DF to be persistent (Hoffman et al., 1995). For fertility to increase, SMB protocol ought to be given between days 8 and 12 of the estrous cycle (Beal, 1998).

Anestrual heifers treated with E_2 in combination with MGA and 200 mg P_4 resulted in 81% estrus synchrony, relative to 66% for MGA alone and 28% for $PGF_{2\alpha}$ (Fike et al., 1999). MGA-treated heifers with higher concentrations of E_2 had higher estrus synchrony (79%), compared to MGA-treated heifers having E_2 less than 5 pg/ml (21%) (Fike et al., 1999). The use of estrogens in combination with progestins, in synchronization regimens, either as long term (13-15 days) or short term (7-9 days) treatment have resulted in variable fertility results (Thimonier et al., 1975). Conception rates with SMB protocol have varied, depending on what day the treatment was initiated (Brink and Kiracofe, 1988). Heifers treated on days 11 or less, yielded 47% CR as opposed to 37% CR for heifers treated on days 12 or more. In another study, Kastelic et al. (1996) found that treatment with E_2 and P_4 on the second day of an 8-d MGA treatment protocol has resulted in improved pregnancy rates (PR) compared to MGA

alone.

The inclusion of estrogens appears to be promising in achieving optimum estrus synchrony as well as fertility rates, especially when used in the CIDR-based protocols, regardless of the stage of the cycle. For inconclusive reasons, the use of estrogens in progestin treatments such as SMB and MGA seems to be influenced by the day the treatment is given. When such treatments are given late in the cycle, fertility is lower, much like when norgestomet or MGA is given alone.

2.14. $PGF_{2\alpha}$ in synchronization

Prostaglandin $F_{2\alpha}$ has been used to synchronize estrus, as a luteolytic agent that is responsible for the demise of the functional CL (Lauderdale, 1972). Rowson et al. (1972) reported ineffectiveness of 0.5 mg/ml $PGF_{2\alpha}$ given between days 1-4 of the estrous cycle, in contrast to treatment given between days 5-16, which resulted in estrus three days after treatment. On average, cattle with functional CL's come into estrus within 60 to 72 h following $PGF_{2\alpha}$ injection (Beal, 1998). The effect of $PGF_{2\alpha}$ on synchronized estrus varies throughout the estrous cycle. Longer and more variable intervals to estrus and ovulation were observed for heifers treated with $PGF_{2\alpha}$ treatment on day 12 compared with treatment on days 5 to 8 or days 15 and 16 of the estrous cycle (Kastelic, 1994; De Rensis and Peters, 1999). In a three-wave cycle, the peak of DF development may coincide with days 5-8 and 15 to 16 of the estrous cycle. Prostaglandin $F_{2\alpha}$ has been used in single-injection protocols as well as two-injection protocols.

2.14.1. Single injection

The single-injection protocol either involves $PGF_{2\alpha}$ injection prior to estrus detection by 5 days (Lauderdale et al., 1980), or 5 days after breeding (4 days of estrus detection) (Moody, 1979). For unknown reasons, a single injection of $PGF_{2\alpha}$ does not cause luteolysis before day 5 of the estrous cycle (Lauderdale, 1972; Kiracofe et al., 1985). Large luteal cells (the site of synthesis of $PGF_{2\alpha}$) are almost non existent in the day 4 CL, but as the estrous cycle progresses (days 8 and 12) the number of LLC increased (Fitz et al., 1982). Further, it was suggested (Niswender et al., 2000) that $PGF_{2\alpha}$ down-regulates mRNA encoding cyclooxygenase in bovine CL, early in the estrous cycle, which coincides with the time when $PGF_{2\alpha}$ cannot cause luteolysis, and suggesting that local synthesis of $PGF_{2\alpha}$ may be important in this process. Cows must be heat detected and bred over 10 days if cows are given a single $PGF_{2\alpha}$ injection on day 5 of the estrous cycle (Kastelic et al., 1996). Higher PR was observed in cows treated with a single $PGF_{2\alpha}$ injection relative to control cows (Moody, 1979).

2.14.2. Double injections

The rationale behind using a double $PGF_{2\alpha}$ injection protocol was to overcome the circumstance when cows/heifers are not responsive to $PGF_{2\alpha}$ prior to day 5 of the estrous cycle. The two-injection $PGF_{2\alpha}$ protocols are usually given 11 to 14 days apart in heifers (Macmillan et al., 1978) and cows (Peters and Ball, 1987; Stephens and Rajamahendran, 1998). To achieve this recommended period (11 or 14 day intervals) animals must be between days 7 to 17 of the luteal phase of the estrous cycle (Nebel and Jobst, 1998). Stephens and Rajamahendran (1998) found that the two-injection $PGF_{2\alpha}$

treatment 11 days apart resulted in 83% and 90% synchrony (within 72 h, after treatment) in two experiments, similar to that reported by Twagiramungu et al. (73%; 1995). Fertility was found high and similar to that of control, as evidenced by the study of Moody and Lauderdale (1977) who reported 59% conception rate after $PGF_{2\alpha}$, relative to 62% in cows bred after a naturally occurring heat. Lucy et al. (1986), however, reported that CR following one fixed timed artificial insemination (TAI) at 80 h after the second of two injections of $PGF_{2\alpha}$ (23%) was less than that detected in similarly treated cows (51%) inseminated at estrus.

The onset of estrus in cows following $PGF_{2\alpha}$ has been variable with a significant proportion of cows showing estrus outside the range of fixed TAI. The variation in the timing of estrus is probably caused by differences among cows in the rate of CL regression following treatment. Also, variation in the timing of ovulation of the DF following usage of $PGF_{2\alpha}$ results in lower fertility when animals are TAI (Beal, 1998).

2.15. $PGF_{2\alpha}$ and progestins in synchronization

Shortening the period of progestagen treatment proved to be an important factor in achieving higher fertility rates (Roche, 1974). To reduce the effect of long exposure of progestins following treatment, $PGF_{2\alpha}$ was used at or after progestin treatment (Wishart and Young, 1974; Heersche et al., 1974). Norgestomet implant with $PGF_{2\alpha}$ injection prior to or at the removal of implant was the protocol used by these scientists. Smith et al. (1984) included PRID for 7 days and an injection of $PGF_{2\alpha}$, one day before the end of PRID treatment. Beal and Good (1986) proposed a short term feeding of MGA combined

with an injection of $PGF_{2\alpha}$ at the end of the MGA feeding. This protocol resulted in good synchrony but lower fertility, relative to untreated cows (Beal et al., 1988). Beef heifers showed estrus within 5 days after $PGF_{2\alpha}$ injection which was given 6 or 7 days following progestagen implantation (Heersche et al., 1979). To achieve higher fertility rates, MGA was fed for 14 days followed by a $PGF_{2\alpha}$ injection 17 days later (Odde, 1990). This treatment yielded higher estrus synchrony when compared to a single-injection $PGF_{2\alpha}$ treatment (Patterson et al., 1995), and lower synchrony relative to SMB (Brown et al. 1988). With this protocol, animals came to estrus 24 h after the termination of the treatment (King et al., 1994). Fertility rates were comparable to the single-injection $PGF_{2\alpha}$ protocol (Patterson et al., 1990; Jaeger et al., 1992). However, it should be noted that this protocol is impractical due to its long duration.

The interval from $PGF_{2\alpha}$ treatment and its luteolytic effect of the CL to expression of estrus is determined by the stage of development of the DF at the time of treatment, which in turn affects PR following fixed TAI. Heifers with viable DF returned to estrus 48 to 60 h after $PGF_{2\alpha}$, whereas, heifers exhibited estrus in 5 to 7 days when the DF had started to undergo atresia (Savio et al., 1990). The DF in the first wave loses its ability to ovulate after prostaglandin-induced luteolysis when it has reached the late plateau or regression phase of its development (Fortune et al., 1991). If luteolysis is induced before the mid-static phase of a DF, the follicle will ovulate, resulting in a relatively short interval from treatment to ovulation (Kastelic et al., 1990). If luteolysis is induced after the mid-static phase of the DF, the DF of the next wave will grow and become the ovulatory follicle, resulting in a longer interval from treatment to ovulation (Kastelic et

al., 1990).

The variable results in terms of time of estrus, ovulation, timed insemination, and fertility following the synchronization treatments cited above, clearly indicated the need to develop synchronization protocols based on the sound understanding of both follicular and CL dynamics, and which enable single TAI and improve PR.

V. Estrus synchronization protocols based on elimination of the dominant follicle and induction of new follicular wave

Non-hormonal treatments such as follicle ablation and hormonal treatments such as LH, hCG, GnRH, E_2, and P_4, have been used to remove the suppressive effect of the DF on the SF's and initiate new follicular waves. However, the most common treatments for altering follicular turnover in conjunction with estrus synchronization have been the use of GnRH analogs (Twagiramungu et al., 1995; Pursley et al., 1995) or the use of E_2 in conjunction with progestins (Bo et al., 1994; Martinez et al., 2000).

2.16. Follicle ablation

Removal of the DF by means of electrocauterization has been shown to result in emergence of new follicular wave (Adams et al., 1993). Bergfelt et al. (1994) used transvaginal aspiration of follicles (> 5 mm in diameter) as means of ablation, with an injection of $PGF_{2\alpha}$, and found that a new follicular wave was initiated 2 days after ablation. Further, ovulation rates were higher (81%) in the ablation group than control (47%) (Bergfelt et al., 1994).

2.17. LH

Lulai et al. (1994) used porcine LH (pLH) in pregnant heifers to induce ovulation (100%) of the DF in pregnant heifers. Also, Martinez et al. (1999) reported 78% overall ovulation in heifers injected with LH on days 3, 6 and 9 of the estrous cycle. However, only heifers that ovulated had new wave emergence as a result of LH with ovulation occurring 1.3 days after treatment (Martinez et al., 1999). The effect of LH was mostly on follicles that were in the late-growing or early-static stages (Martinez et al., 1999), which acquire LH receptors on granulosa cells (Xu et al., 1995; Bodensteiner et al., 1996; Beg et al., 2001).

2.18. hCG

The proportion of three-wave cycle increased over two-wave cycle after administration of hCG to cows (Sianangama and Rajamahendran, 1996). The effect of hCG on the DF is most effective between days 5 and 7 of the estrous cycle (Rajamahendran and Sianangama, 1992; Schmitt et al., 1996).

2.19. GnRH

The administration of GnRH analogs stimulates luteinization or ovulation of medium and large follicles, which results in recruitment and selection of a new DF from a new follicular wave (Thatcher et al., 1989; Macmillan and Thatcher, 1991; Rettmer et al., 1992; Wolfenson et al., 1994; Twagiramungu et al., 1994; Pursley et al., 1995; Kastelic and Mapletoft, 1998; Vasconcelos et al., 1999). The effectiveness of GnRH treatment is influenced by the stage of follicular development at the time of treatment (Pursley et al.,

1995; Twagiramungu et al., 1995; Vasconcelos et al.,1999; Moreira et al., 2000). Follicles in the growing phase (day 3.8) respond more to GnRH, thus ovulation, than follicles in the static (day 7.4) or regressing (day 12.7) phases (Silcox et al., 1993; Schmitt et al., 1996; Wiltbank, 1997; Martinez et al., 1999). Treatment with GnRH followed by $PGF_{2\alpha}$ 6 or 7 days later, increased the number of heifers showing estrus within five-day period, and improved the precision of synchrony two to three days after $PGF_{2\alpha}$ injection, compared to heifers receiving $PGF_{2\alpha}$ alone (Thatcher et al., 1989; Twagiramungu et al., 1992). Further, Roy and Twagiramungu (1999) detected 51.3% behavioral estrus in cows treated with GnRH and $PGF_{2\alpha}$ administered 7 days later. However, Stephens and Rajamahendran (1998) found 22 of the 30 heifers showing standing heat (73%) when treated with GnRH followed by $PGF_{2\alpha}$, 7 d later, compared to 83 % of heifers treated with $PGF_{2\alpha}$ alone. The 7 day interval between GnRH and $PGF_{2\alpha}$ resulted in better synchronization rate and PR compared to those treated with injections of $PGF_{2\alpha}$ 11 days apart (Thatcher et al., 1993; Twagiramungu et al., 1994). However, Stephens and Rajamahendran (1998) found PR to be 40% and 62% for GnRH + $PGF_{2\alpha}$, and $PGF_{2\alpha}$ given 11 days apart, respectively. Premature occurrence of estrus in animals administered GnRH during the late luteal phase could be one fault of the GnRH + $PGF_{2\alpha}$ treatment (Pursley et al., 1995; Vasconcelos et al., 1999; Moreira et al., 2000).

2.20. Estradiol

Estradiol induces follicle atresia (Hutz, 1988; Diershke et al., 1994). It has different effects on the DF, depending on when estradiol is administered. Estradiol causes regression of the DF when it is given during the growing phase of the follicle, but not

during the middle and end growing phases of the follicle (Bo et al., 1993). Regression of the growing DF stimulated FSH surge and initiation of a new follicular wave; however, the regression of a static or regressing follicle witnessed both delay in FSH surge and start of new follicular wave (Bo et al., 1993). The latter authors attribute the delay in FSH surge to the long half-life of valerate used with estradiol in their experiment. When E_2 was given during the mid-growing phase of the DF, there was no delay in initiation of new follicular wave (Bo et al., 1993). Emergence of a second follicular wave was earlier in heifers treated with E_2 on day 3 than day 6 of the estrous cycle (Bo et al., 1995). Also, in the same report, when E_2 was administered on day 9 of the cycle, follicles of the second follicular wave were suppressed, and a third wave was initiated. Estradiol-17β administered early in the luteal phase (in the absence of high P_4 concentration) did not regress the DF and caused a surge of LH (Short et al., 1979; Bo et al., 1993; Bo et al., 1994).

2.21. Progestins

Exogenous as well as endogenous P_4 act to prevent E_2-induced LH surge in cattle with or without a functional CL (Scaramuzzi, 1971; Hobson and Hansel, 1972; Abeyawardene and Pope, 1987; Stock and Fortune, 1993; Bo et al., 1994). Implanting progesterone on day 17 of the estrous cycle blocked ovulation (Roche, 1974). Time of PRID insertion in heifers determined the quality of embryos (Goulding et al., 1994). For example, poor quality embryos were detected after superovulation when PRID was inserted in the early luteal phase, and was attributed to dominance of the DF over SF.

A growing DF can be suppressed by exogenous P_4 in a dose-dependent manner, whereas, static or regressing DF do not (Adams et al., 1992a). Progesterone may have an indirect effect on FSH concentrations by causing an early regression of the growing-phase DF, which can result in an early FSH surge and early emergence of follicular wave (Adams, 1994).

2.22. Estradiol and progestins

Treatment with P_4 and estradiol together consistently causes regression of the large follicle, stimulates emergence of a new follicular wave 3-5 days after estradiol injection, and blocks LH surge and ovulation regardless of the stage of the estrous cycle (Price and Webb, 1988; Bo et al., 1994; Bo et al., 1995; Garcia and Salaheddine, 1996). Studies by our laboratory found that treating heifers with P_4 and E_2 regressed persistent follicles, in contrast to cows treated with norgestomet alone (Taylor et al., 1993; Rajamahendran and Manikkam, 1994). Heifers treated with E_2 in combination with SMB had smaller DF diameter than heifers treated exclusively with E_2 (Bo et al., 1994). Studies were conducted to examine whether $E_2 + P_4$ treatment in conjunction with a CIDR device would minimize variation in the interval from treatment to ovulation. Treatment of beef heifers with CIDR for 7 days, plus intramuscular treatments of 100 mg P_4 and 5 mg E_2 at the time of CIDR insertion and 500 μg cloprostenol at the time of CIDR removal, resulted in 75% of heifer ovulating between 72-84 h after CIDR removal, compared to 40% of heifers treated with two injections of $PGF_{2\alpha}$ 11 days apart, and 33% of heifers treated with CIDR alone (Bo et al., 1994). The suppressive effect of E_2 and P_4 together was attributed to the suppression of both FSH and LH secretion (Bo et al., 1994). Lago et

al. (1999) compared PR and CR between three estrus synchronization protocols; CIDR (plus estradiol benzoate and P_4), two injections of $PGF_{2\alpha}$ (14 days apart) and natural estrus. They concluded that CIDR yielded the highest PR and CR (83% for both) relative to two injections of $PGF_{2\alpha}$ (64% and 69%) and natural estrus (71% and 79%). In agreement with these results, Hamilton et al. (Ontario Beef Research Update; 98/99) reported CR between 60%-95%, in the CIDR-containing synchronization treatments.

VI. Synchronization protocols based on emergence of new follicular wave, synchronization of ovulation/timed artificial insemination (TAI)

Maximizing estrus detection rates can improve overall reproductive efficiency in cattle. Henricks et al. (1971) reported that peak estrus activity often occurs at night and determination of the actual onset of standing estrus may be difficult without 24 h observation, which causes difficulties in terms of controlling the time of estrus, and requires copious amounts of time and labor. Therefore, protocols such as Ovsynch and CIDR + estradiol protocols were developed, which are based on: a) elimination of the DF, b) initiation of a new follicular wave, and c) synchronization of ovulation and TAI.

2.23. Ovsynch ovulation synchronization/TAI protocol

The Ovsynch ovulation synchronization protocol developed by Pursley et al. (1994, 1995) consists of two GnRH injections and a $PGF_{2\alpha}$ injection. The first GnRH injection is administered irrespective of the stage of the estrous cycle, followed by a $PGF_{2\alpha}$ injection 7 days later (to regress either the old or new formed CL), and a final GnRH injection 48 h after the $PGF_{2\alpha}$ treatment. The first GnRH injection is intended to

either induce ovulation or luteinization of the DF, to initiate a new follicular wave. The second GnRH injection is given to stimulate LH surge comparable to the spontaneous preovulatory LH surge, thus synchronizing ovulation (between 24-32 h after second GnRH injection; Pursley et al., 1995). Timed insemination following Ovsynch takes place 16 h after the second GnRH injection (Pursley et al., 1995). This protocol is more consistent in terms of synchronized ovulation in cows than heifers. Only 18 of 24 heifers ovulated between 24 and 32 h after the second injection of GnRH, compared to 20 of 20 cows (Pursley et al., 1995). Turnover of follicular waves is more rapid in heifers than lactating cows, increasing the probability of administering the first GnRH injection in the absence of a DF and/or turning over a follicular wave before ovulation can be induced with a second GnRH injection (Haughian and Wiltbank, 2002). This might explain the low ovulation rate (of heifers) to the first GnRH injection (from 30 to 54%; Pursley et al., 1995; Castilho et al., 2000; Ambrose et al., 2001), which might have resulted in low synchronization rate and PR (Pursley et al., 1995; Twagiramungu et al., 1995; Pursley et al., 1997a). In agreement with these studies, Martinez et al. (1999) stated that the first GnRH does not always induce ovulation, and a new follicular wave was induced (56%) only when ovulation occurred in response to treatment. In addition, 6% of cows ovulated before the second injection of GnRH (Vasconcelos et al., 1999). All cows that ovulated before the second GnRH injection were in the late estrous cycle, and thus had not ovulated following the first GnRH injection (only 36% ovulated) (Vasconcelos et al., 1999). The same authors suggested that these cows would be expected to have normal luteal regression prior to $PGF_{2\alpha}$ treatment, because they were in the late estrous cycle and did not have newly formed CL; also, the follicle that did not respond to the first GnRH

injection, might be expected to have grown significantly to cause behavioral estrus prior to the second GnRH injection. Moreira et al. (2000) found that when the Ovsynch protocol was initiated on day 2 of the estrous cycle, the spontaneous growth of a new follicular wave had already started. Because a new follicular wave was not initiated in response to the first GnRH injection, the DF reached a plateau phase sooner and was possibly undergoing early stages of atresia when the second GnRH injection was given. These authors concluded that the starting day of the Ovsynch/fixed TAI protocol at metestrus might have compromised the quality of the preovulatory follicle and subsequent competence of the oocyte. Therefore, ovulation to the first GnRH injection is imperative in the Ovsynch protocol, especially for cows in the late or early estrous cycle. The overall ovulation synchronization rate (cows that ovulated to second GnRH) varied in terms of their response to the first GnRH (92% if ovulation to first GnRH vs. 79% if no ovulation) (Vasconcelos et al., 1999). Compared to PR observed following AI at natural estrus (52%, 47% and 70%; Pursley et al., 1995 and Stevenson et al., 1996, and Pursley et al., 1997b, respectively), PR following Ovsynch and fixed TAI averaged about 40% in lactating dairy cows (Pursley et al., 1997a,b; Schmitt et al., 1996; Ambrose et al., 1999; Ambrose et al., 2000), and dairy heifers (Schmitt et al., 1996; Pursley et al., 1997a; Stevenson et al., 2000). To date, there is insufficient information available on LH profiles, CL function and follicular dynamics following Ovsynch ovulation synchronization protocols.

2.24. CIDR-based ovulation synchronization/TAI protocols

Pregnancy rates following CIDR and fixed TAI have averaged 60-80% in beef heifers, beef cows as well as in dairy cows (Burke et al., 2000; Day et al., 2000; Martinez

et al., 2000). In these studies, at the time of CIDR insertion, cattle were usually injected with E_2 (to regress the DF) and P_4 (to act as artificial CL if CL is not present and block LH surge and ovulation), to initiate a new follicular wave; $PGF_{2\alpha}$ was given at CIDR removal (inserted for 7 days) to cause luteal regression, and a second E_2 injected (24 h after $PGF_{2\alpha}$/CIDR removal) to synchronize ovulation for TAI. The CIDR device resulted in an increase in plasma P_4 concentrations 2 h following the time of insertion (Burke et al., 1999). The same authors reported that P_4 levels did not increase beyond days 3 to 5 of insertion of CIDR device. Because the CIDR protocol is normally initiated regardless of the stage of the estrous cycle, the possibility of animals with small luteal structure (either early or regressed) exists. Exogenous P_4 is therefore, administered to mimic the mid-luteal phase with regard to circulating P_4 concentrations (Taylor and Rajamahendran, 1994). These latter authors reported that 100 mg of exogenous P_4 resulted in elevation of P_4 concentrations similar to that found during mid-luteal phase (5-6 ng/ml). Therefore, the administration of 100 mg of exogenous P_4 is necessary to block a possible E_2-induced LH surge and ovulation, which could occur as a result of the exogenous E_2 at the start of treatment. Bo et al. (2000) indicated that elevation of E_2 for a period between 12 h (0.5 or 1 mg) to 42 h (5 mg) is necessary to cause atresia of the DF. Elevation of E_2 concentration for 10 h was not sufficient to induce atresia, according to these scientists. Further, the use of 1 mg of estradiol benzoate (EB) was not as effective in inducing follicle suppression when compared to 2 mg of EB (Day et al., 2000). Intramuscular injection of EB has been shown to elevate E_2 concentration to its maximum within 11-15 h (Roche et al., 1999). Higher concentration of E_2 was found as a result of 5 mg of EB compared to either 1.0 or 2.5 mg of EB (Roche et al., 1999). All doses of EB decline FSH

concentration, however the extent of the decline and the time for FSH to return to pretreatment levels is longer after 5 mg EB (Roche et al., 1999). Regardless, the effect of EB on FSH is transitory and FSH starts to increase in the presence of high as well as declining E_2 in circulation (Roche et al., 1999). Estradiol with P_4 can either prevent selection of DF (reduction of FSH) or decrease diameter of DF after selection had occurred without affecting the timing of the next new wave emergence (Roche et al., 1999). The second E_2 injection is given to: 1) reduce variation in estrus timing that may occur following removal of the CIDR device, and 2) stimulate LH surge necessary for ovulation. Researchers have observed enhanced estrus behavior in response to intramuscular EB injection and following P_4 removal (Figueroa et al., 1988). However, information on LH profiles, CL function and follicular dynamics following CIDR ovulation synchronization protocols is scarce.

VII. Rationale and objectives of the thesis

Synchronization of the follicular wave and DF is necessary in ovulation synchronization protocols as a means to overcome variability in time of estrus and ovulation after synchronization. The tight synchrony between TAI and the time of ovulation in turn would enhance fertility, following fixed TAI. Treatments such as the use of GnRH 7 days prior to the use of $PGF_{2\alpha}$, or the use of E_2 in conjunction with P_4, have shown promising results in terms of their effects on the time of emergence of new follicular wave, and elimination of the DF, when treatments are imposed. Further, the inclusion of E_2 following the removal of CIDR and $PGF_{2\alpha}$ administration (CIDR protocol), or GnRH 48 h after $PGF_{2\alpha}$ (Ovsynch protocol) has resulted in a more precise

timing of estrus, LH and ovulation. However, the effectiveness of the Ovsynch protocol differs with regard to the stage of the estrous cycle, unlike the CIDR protocol, which have shown consistent results in terms of stimulating wave emergence and elimination of the DF. The Ovsynch protocol has been shown to be more effective in cows than heifers, whereas CIDR protocols have been shown to be consistent in both heifers and cows. Unfortunately, the limited and available literature on Ovsynch and CIDR treatment protocols focuses mainly on ovarian follicular dynamics during synchronization treatment and ovulation synchronization success. Clearly, further research is warranted to understand LH responses, CL formation and function, ovarian follicular dynamics, and PR following Ovsynch and CIDR treatment protocols for ovulation synchronization.

Therefore, the specific objectives of this thesis were: 1) to compare LH profiles, follicular dynamics, CL function, and PR, following Ovsynch and CIDR ovulation synchronization/TAI protocols (CHAPTER 3); 2) to compare in vitro CL function following treatment with the Ovsynch and CIDR ovulation synchronization protocols (CHAPTER 4); and 3) to compare in vivo CL function following treatment with the Ovsynch and CIDR ovulation synchronization/TAI protocols (CHAPTER 5).

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CHAPTER 3

OVARIAN FOLLICULAR DYNAMICS, LH PROFILES, PROGESTERONE CONCENTRATION, AND PREGNANCY IN DAIRY HEIFERS FOLLOWING OVSYNCH AND CIDR OVULATION SYNCHRONIZATION/TIMED ARTIFICIAL INSEMINATION PROTOCOLS

3.1. Abstract

The objective of this study was to compare ovarian follicular dynamics, luteinizing hormone (LH) profile, and progesterone (P_4) concentrations in dairy heifers following Ovsynch and CIDR ovulation synchronization protocols. Eighteen Holstein dairy heifers were randomly assigned to either Ovsynch (n = 6), CIDR (n = 6), or $PGF_{2\alpha}$ (control, n = 6) treatment protocols. The Ovsynch treatment protocol consisted of an initial injection of gonadotropin-releasing hormone (GnRH; Factrel®, 100 μg), followed 7 days later by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; Lutalyse®, 25 mg) and 48 h later a second injection of Factrel® (100 μg). The CIDR treatment protocol consisted of initial injections of P_4 (100 mg) and estradiol-17β (E_2, 5 mg) and insertion of controlled internal drug release (CIDR-B®, 1.9 g progesterone) followed 7 days later by Lutalyse® (25 mg), 24 h later removal of CIDR, and 48 h after Lutalyse® a second 5 mg injection of E_2. The $PGF_{2\alpha}$ treatment protocol consisted of two injections of Lutalyse® (25 mg each), 12 days apart. Artificial insemination (AI) was performed at 64, 76, and 72 h after $PGF_{2\alpha}$ injection for Ovsynch, CIDR, and $PGF_{2\alpha}$ protocol cows, respectively. Ultrasonography was performed throughout the treatment protocols and following ovulation for one

complete cycle to monitor ovarian follicular and corpus luteum (CL) dynamics. Serial blood samples were taken (starting at 46, 44 and 24 h after $PGF_{2\alpha}$ injection, for Ovsynch, CIDR, and $PGF_{2\alpha}$ treatment protocols, respectively) to determine the LH profile. Blood samples were also collected to determine P_4 concentrations throughout the treatment protocols and for one complete cycle following ovulation. The diameter of the ovulatory follicle (mm) at the second injection was similar ($P > 0.05$) between Ovsynch and $PGF_{2\alpha}$ treatment protocols but larger ($P < 0.004$) than CIDR treatment protocol. Duration of the ovulatory follicle (days) was higher ($P = 0.05$) in the Ovsynch compared to CIDR and $PGF_{2\alpha}$ treatment protocols which were not different. The area under the curve for LH was less ($P < 0.01$) in the $PGF_{2\alpha}$ protocol than Ovsynch and CIDR treatment protocols. The duration of LH surge (h) was not different ($P > 0.05$) between the treatment protocols. The time of the first follicular wave emergence following ovulation was different ($P < 0.0001$). The time of first wave deviation, duration of first wave dominant follicle (DF) and maximum diameter (mm) of the first wave DF were not different ($P > 0.05$) between treatment protocols. Maximum diameter of the second wave DF was higher ($P < 0.05$) in the $PGF_{2\alpha}$ than Ovsynch and CIDR which were not different ($P > 0.05$). The area under the curve for P_4 was similar between treatment protocols ($P > 0.05$) and did not regress significantly on LH area under the curve. However, P_4 area under the curve regressed significantly ($P < 0.05$) and positively ($r = 0.4$) on LH surge duration. Longer duration and larger diameter of the ovulatory follicle in the Ovsynch compared to CIDR protocol did not affect post synchronization LH profiles, follicular dynamics and P_4 concentrations, which were similar between Ovsynch and CIDR ovulation synchronization protocols.

3.2. Introduction

Pregnancy rate (PR) after artificial insemination (AI) is the product of estrus detection and conception rates (Burke et al., 1996) and it can be improved by maximizing estrus detection rates. The use of AI has not been widely adopted by beef producers because estrus detection is time consuming (Small et al., 2001). In addition, low rate of estrus detection (50%) is also considered a main cause of poor reproduction performance in lactating dairy cows (Vasconcelos et al., 1997; De Rensis and Peters, 1999). To improve reproductive management and thus profitability in the cattle industry, various estrus synchronization protocols have been adopted to maximize the use of AI by reducing the time and labor involved in estrus detection. Until recently, methods that have been used to control and further improve synchronization were aimed at either extending the luteal phase by the use of progestagens (Taylor and Rajamahendran, 1991; Anderson and Day, 1998) or shortening the luteal phase and consequently, the estrous cycle by the use of one of the more common methods of synchronizing estrus in cattle, two injections of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) administered 11 to 14 days a part in heifers (Macmillan et al., 1978) and cows (Stephens and Rajamahendran, 1998). However, maturity of the dominant follicle (DF) and thus ovulation varies with individual cows, and thus estrus cannot be punctually synchronized with these synchronizing protocols. A 7-day period of estrus detection is necessary following administration of $PGF_{2\alpha}$ 11 to 14 days a part (Lucy et al., 1986; Stephens and Rajamahendran, 1998). As a result, timed AI (TAI) 72 to 80 h after a second $PGF_{2\alpha}$ injection resulted in significantly lower PR than animals inseminated after natural estrus (Stevenson et al., 1987; Stevenson et al., 1989). The reduced fertility rates obtained following the earlier protocols made it necessary to

understand ovarian follicular and corpus luteum (CL) dynamics in cattle. Ovsynch and CIDR are emerging protocols that are aimed to synchronize both the development of the follicle and ovulation of that follicle (Hirad et al., 1999; Martinez et al., 2002). These protocols are also aimed to facilitate AI of cattle at a fixed time. The Ovsynch protocol uses an initial injection of gonadotropin releasing hormone (GnRH) to synchronize follicular wave emergence and induce or maintain a CL that is responsive to $PGF_{2\alpha}$ 7 days later. At 48 h after administration of $PGF_{2\alpha}$, ovulation is synchronized by a second injection of GnRH (Pursley et al., 1995; Hirad et al., 1999; Peters and Pursley, 2003). The CIDR protocol uses an initial injection of progesterone (P_4) and estradiol and an insertion of an intravaginal P_4 device (CIDR-B®) to initiate a follicular wave and maintain P_4 levels until injection of $PGF_{2\alpha}$ 7 days later. The CIDR-B® device is then removed and an another estradiol injection is administered following a $PGF_{2\alpha}$ injection to synchronize ovulation (Martinez et al., 2000; Ambrose et al., 2001). (See chapter 2 for details on Ovsynch and CIDR ovulation synchronization protocols).

One aim of ovulation synchronization protocols that are based on timed insemination is to control or advance the time of ovulation. The tight synchrony between TAI and the time of ovulation in turn enhances CL function and fertility. Therefore, the inclusion of a second injection of estradiol following the removal of CIDR-B® (CIDR protocol), or GnRH 48 h after injection of $PGF_{2\alpha}$ (Ovsynch protocol) has resulted in a more precise timing of estrus, luteinizing hormone (LH) and ovulation (Pursley et al., 1995; Hanlon et al., 1997; Lammoglia et al., 1998). A spontaneous LH surge can last between 6-10 h (Chenault et al., 1975; Walters and Schallenberger, 1984), whereas

GnRH-induced LH remains elevated for up to 6 h (Johnson and Reeves, 1988; Rajamahendran et al., 1998). Rajamahendran et al. (1998) also reported increased P_4 secretion between days 12 and 21 in cows treated with implants containing 700 and 2100 μg Doslorelin (GnRH agonist). These authors attributed these findings to an acute and chronic release of LH, which resulted in a more differentiated and functional accessory corpora lutea. Further, it has been found that estradiol-induced LH surge remains elevated for 8 h (Beck and Convey, 1977; Hanlon et al., 1997), which is comparable to the spontaneous LH surge duration.

Transrectal ultrasonography has provided an efficient tool for studying ovarian dynamics (i.e. follicle and CL development) in cattle, and confirmed that follicular growth occurs in wave-like patterns (Rajamahendran et al., 1994). Most cattle either have two or three follicular waves during the estrous cycle, with the DF being the ovulatory follicle at the time of CL regression (Rajamahendran and Walton, 1988; Ginther et al., 1989). Ultrasound imaging revealed that follicular development occurred in two waves in normally cycling heifers and dairy cows (Pierson and Ginther, 1987; Burke et al., 1999). Follicle turnover has been reported to be more rapid in heifers than in cows (Pursley et al., 1995; Pursley et al., 1997a). In a three follicular-wave cycle, waves begin around days 2, 9, and 16, whereas in a two follicular wave cycle, waves start around days 2 and 11 (Sirois and Fortune, 1988).

Processes such as the length of the estrous cycle, ovulation, as well as the fate of the embryo, may all depend on the function of the CL (Milvae et al., 1996). Inadequate

CL function, also called luteal dysfunction, is characterized by an estrous cycle of normal duration and low peripheral P_4 (Lamming et al., 1981). Cows with low P_4 concentrations between days 10 and 16 post-breeding did not maintain pregnancy (Henricks et al., 1971; Lamming et al., 1989). Pregnancy rates in heifers following synchronization using the Ovsynch protocol were reported (Ambrose et al., 2000; Small et al., 2001) to be considerably lower (40%) than what could be achieved with breeding at detected estrus (≤ 70%) (Pursley et al., 1997a). However, PR of 60-80% have been reported in heifers following synchronization with CIDR protocols (Martinez et al., 2000; Ambrose et al., 2001; Martinez et al., 2002).

The LH profile prior to ovulation has been shown to determine ovulation (Rajamahendran et al., 1989), formation and function of the CL (Rajamahendran et al., 1998), ovarian follicular dynamics (Ireland et al., 2000), and therefore PR (Schweiger et al., 1989). However, very little information is available on LH profiles, the use of ultrasound in monitoring follicular development and CL function following synchronization with CIDR and Ovsynch ovulation synchronization protocols. Therefore, the objectives of this study were to compare and examine 1) LH profiles, 2) follicular dynamics 3) CL function, and 4) PR, following Ovsynch and CIDR ovulation synchronization/TAI protocols.

3.3. Materials and Methods

3.3.1. Animals and treatments

This study was carried out at The University of British Columbia South Campus Dairy Research & Training Complex, Vancouver, Canada. Eighteen Holstein heifers (average age = 15 months; average weight = 330 kg) were maintained under the same nutritional and management conditions. Animals, were assigned randomly to three different treatment protocols, Ovsynch (n = 6), CIDR (n = 6), and $PGF_{2\alpha}$ (n = 6).

3.3.2. The Ovsynch protocol

The Ovsynch treatment protocol (Figure 3.1a) consisted of an initial injection of GnRH (100 μg i.m. of Factrel®; Fort Dodge Laboratories, Fort Dodge, IA), followed 7 days later by an injection of $PGF_{2\alpha}$ (25 mg i.m. of Dinoprost; Lutalyse®, Pharmacia & Upjohn, Orangeville, ON) and 48 h later by a second injection of 100 μg i.m. GnRH (day -1). Artificial insemination (day 0) was carried out at 64 h after the $PGF_{2\alpha}$ injection.

3.3.3. The CIDR protocol

The CIDR treatment protocol (Figure 3.1b) consisted of initial injections of P_4 (100 mg; Sigma-Aldrich Canada Ltd) and estradiol-17β (E_2, 5 mg; Sigma-Aldrich Canada Ltd) and insertion of CIDRs (CIDR-B®; 1.9 g progesterone; Inter-Ag, Hamilton, NZ) followed 7 days later by an injection of $PGF_{2\alpha}$, (25 mg), removal of CIDR-B® 24 h later, and a second 5 mg injection of E_2 (day -1) 48 h after the $PGF_{2\alpha}$ injection. Artificial insemination (day 0) was carried out at 76 h after the $PGF_{2\alpha}$ injection.

3.3.4. The $PGF_{2\alpha}$ protocol

Heifers were synchronized with two 25 mg $PGF_{2\alpha}$ injections, 12 days apart (Figure 3.1c). Artificial insemination (day 0) was carried out at 72 h after the second $PGF_{2\alpha}$ injection.

3.3.5. Blood sampling and radioimmunoassay for LH

In the Ovsynch treatment protocol, initial blood sampling for LH was performed at 2 h intervals starting 2 h before and ending 16 h after the second GnRH injection. Thereafter, blood was sampled at 4 h intervals until 56 h after the second GnRH injection. In the CIDR treatment protocol, heifers were sampled at 4 h intervals starting from 4 h before until 16 h after the second E_2 injection. Then, blood was sampled at 2 h intervals for 16 h (until hour 32), when thereafter collection started at 4 h intervals until 60 h after the second E_2 injection. In the $PGF_{2\alpha}$ treatment protocol, blood sampling started at 24 h after the second $PGF_{2\alpha}$ injection (Rajamehendran et al., 1989) at 4 h intervals for 48 h. Thereafter, collection continued at 2 h intervals until 58 h, and finally at 4 h intervals until 90 h. Blood samples were collected in heparinized tubes and centrifuged at 1000 x g at 4 °C for 20 min. Following centrifugation, plasma was stored at - 20 °C until thawed and analyzed for LH concentrations. Plasma LH concentrations were measured by means of a double antibody radioimmunoassay procedure as reported by Sanford (1987). The sensitivity of the assay was at 0.14 ng/ml. Intra-assay and inter-assay coefficients of variation were assessed from samples of low, intermediate, and high LH concentration in single or multiple assays and were both 5.8%.

3.3.6. Blood sampling and radioimmunoassay for P_4

Blood samples (by coccygeal venipuncture into heparinized tubes) in the Ovsynch and CIDR protocols were taken on days -10, -8, 6, -3, -1, 0, 1, 2, and every other day until day 22 post AI for determination of P_4 profiles. With regard to the $PGF_{2\alpha}$ treated heifers, blood was sampled on days -15, -13, -11, -9, -7, -5, -3, -1, 0, 1, 2, and every other day until day 22 post AI. Immediately following collection, blood was centrifuged at 1000 x g for 20 min and the plasma was then aspirated and stored at - 20 °C until the time of radioimmunoassay. Measurement of P_4 was conducted using a commercially available solid-phase RIA kit (Coat-A-Count; Diagnostic Products Corp., Los Angeles, CA). The kit had previously undergone validation process in our laboratory for measurement of progesterone in cow's milk and plasma (Rajamahendran and Taylor, 1990). On the day of assay an aliquot of 100 µl of the standards was transferred into duplicate appropriate antibody coated tubes labeled A, B, C, D, E, F, and G. The standard tubes (A through G) corresponded to P_4 concentrations of 0, 0.1, 0.5, 2.0, 10.0, 20.0, and 40.0 ng/ml. Then, P_4-buffered I^{125} -labeled P_4 (1.0 ml) was added to all tubes. The tubes were then mixed and incubated at room temperature for 3 h for equilibrium. After incubation, the tubes were decanted and counted for 1 min using the gamma counter (Packard Auto gamma 500, Packard Instruments, Downers Grove, IL, USA). The sensitivity of the assay was at 0.02 ng/ml and the intra-assay and inter-assay coefficient variations were 9.3% and 8.5%, respectively based on three assays.

3.3.7. Ultrasonography

The ovaries of heifers were monitored using a transrectal scanner with a real-time ultrasound scanner equipped with a 7.5 MHz linear array transducer (Aloka Co., Wallingford, CT, USA). The scanning for the Ovsynch or CIDR was carried out on days -10 (start of treatment), -8, -6, -3 (day of $PGF_{2\alpha}$ injection), -1 (day of second injection of GnRH or E_2 of Ovsynch and CIDR, respectively), twice from day 0 (day of AI) until ovulation was confirmed, and every other day until day 22 following AI. For the $PGF_{2\alpha}$ protocol, scanning commenced on days -15 (day of first $PGF_{2\alpha}$ injection), -13, -11, -9, -7, -5, -3 (day of second $PGF_{2\alpha}$ injection), twice starting 24 h after the second injection until ovulation was confirmed. Thereafter, scanning was conducted every other day until day 22 after AI. Scanning for pregnancy was conducted either on days 28 or 35 post AI, and later confirmed by rectal palpation at day 60 post AI. Time of ovulation, day of emergence of follicular wave, time of deviation, diameter of DF and CL, were all evaluated and later analyzed. The disappearance of the DF and the appearance of at least 3-6 small (4 mm) or medium follicles (5 mm) approximated the time of emergence of a new follicular wave. Large follicles were those larger than 10 mm in diameter. Follicle and CL sizes were measured as described previously (Rajamahendran and Walton, 1988; Sirois and Fortune, 1988). Briefly, a ruler calibrated against the scale of the scanner after freezing the image directly on the video screen was used to determine the size of both the follicle and CL sizes. The measurement was based on the spherical shape of these structures. If however, the shape was not spherical, the diameter was calculated by averaging the longest and shortest diameters. These measurements were applied specifically to large follicles (> 10 mm), medium follicles (5 to 9 mm), and the CL(s).

3.4. Statistical analysis

Least-square analysis of variance with repeated measures (JMP® Version 4.1. statistical software, SAS Institute, Cary, North Carolina) was used to analyze follicle and CL diameters, LH area under the curve and LH surge duration, and P_4 area under the curve, over the blood sampling periods, between the Ovsynch, CIDR, and $PGF_{2\alpha}$ treatment protocols. For CL diameter and P_4 area under the curve, LH area under the curve and LH surge duration were added as covariates. Student-t test was used to compare differences between means with significant difference. Treatment effect, heifer within treatment, hour and/or day, and treatment (x) day interaction, all are part of the analysis. To determine a basal LH concentration, the mean LH and standard deviation (SD) for the last six observations for each cow in each treatment protocol were tabulated and basal concentrations were any concentration that fell within three SD of the mean. The Trapezoidal rule was used to calculate the areas under the curves for LH and P_4 (Swokowski et al., 1994). Contingency tables in the chi-square analysis were used to compare: 1) ovulation synchronization rate (responder rate; based on $P_4 < 1$ng/ml on day of AI and > 1ng/ml 6 days post AI), and 2) the overall PR within Ovsynch, CIDR, or $PGF_{2\alpha}$ protocol. The level of significance was set at $\alpha = 0.05$.

3.5. Results

3.5.1.Follicular dynamics and CL function during synchronization

3.5.1.1. The Ovsynch protocol

All six heifers in the Ovsynch protocol had large follicles (12.3 ± 1.8 mm) at the time of the first GnRH injection (Table 3.1). However, five of the six heifers did not

respond to the first GnRH injection. Two of these heifers had large follicles 10.5 and 11.0 mm in diameter, medium follicle (9mm, which later became dominant at the time of second GnRH injection), CL of 23.5 and 27mm in diameter and P_4 concentrations of < 1 ng/ml. This indicates that the DF's at the time of the second GnRH injection in these heifers were as a result of a follicular wave that was already underway. In another heifer, the first GnRH injection apparently was given around the time of emergence of the second follicular wave, evident by the presence of large CL (25 mm in diameter), P_4 levels of 5.0 ng/ml, large follicle (13.5 mm), and presence of five medium follicles. One of the medium follicles later became the DF at the time of the second GnRH injection. The final two of the non-responder heifers (probably at late diestrus) had large CL (29 mm) and high P_4 concentrations (8 ng/ml) at the time of first GnRH injection. One of these heifers had a large follicle (13 mm) and the other heifer had two large follicles (12.7 and 12.0 mm). Progesterone concentrations in these heifers decreased from 9.6 (1^{st} GnRH injection) to < 2.0 ng/ml at the time of $PGF_{2\alpha}$ injection. One of these heifers had synchronized ovulation (ovulated within 48 h after the second GnRH injection), while the other heifer ovulated before the injection of the second GnRH. The heifer that ovulated in response to the first GnRH injection had a CL diameter of 28 mm and the P_4 concentration of 1.0 ng/ml, which indicates that the CL was undergoing luteolysis and the first GnRH injection was administered at later stages of the luteal phase.

3.5.1.2. The CIDR protocol

Similar to the heifers subjected to the Ovsynch protocol, all six heifers in the CIDR protocol group had large follicles (11.53 ± 2.0 mm) at the start of treatment (Table

3.1). Four of these heifers had CL between 10 and 21 mm in diameter, P_4 levels between 2.0 and 5.2 ng/ml, and 9-15 small follicles at the start of the treatment. This suggests that these heifers had already ovulated and started the first follicular wave. The remaining two heifers apparently were at mid-luteal phase when treatment was initiated, as denoted by CL of 25 mm in diameter and P_4 concentrations of 9.8 and 5.4 ng/ml at the start of treatment. The large follicles (13 mm in diameter) of these heifers regressed within 3-4 days of the first E_2 injection. Moreover, P_4 concentrations in these heifers were halved at the time of $PGF_{2\alpha}$ injection, but this did not affect their synchronized ovulation (ovulation after the second E_2 injection).

3.5.1.3. The $PGF_{2\alpha}$ protocol

Five of the six heifers had large follicles (12.6 ± 4.3 mm) at the time of the first $PGF_{2\alpha}$ injection (Table 3.1). However, like in the Ovsynch and CIDR treatment protocols, heifers were at different stages of the estrous cycle when treatment started. Two heifers had CL between 21 and 28 mm in diameter, P_4 concentrations of less than 1 ng/ml, and large follicles of 15 and 19 mm in diameter at the time of the first $PGF_{2\alpha}$ injection. This indicates that these heifers were in late luteal phase of the estrous cycle. Ovulation occurred within 1 to 4 days following the first $PGF_{2\alpha}$ injection. Three heifers were in the second follicular wave and at mid-luteal phase of the estrous cycle at the time of the first treatment of $PGF_{2\alpha}$. This was evident by the presence of large CL (29 mm in diameter), P_4 concentrations between 6-10 ng/ml and large follicles between 10-16 mm in diameter. Ovulation in these heifers occurred within 4 to 6 days of the first $PGF_{2\alpha}$ treatment. The heifer, which lacked a large follicle had a CL (16 mm in diameter), P_4

concentration of less than 1 ng/ml and eight medium follicles. This indicates that the first follicular wave of the estrous cycle had already started, and evident by the lack of response of CL to the first $PGF_{2\alpha}$ injection.

Diameter (mm) of the largest follicle at first injection of GnRH, E_2 and $PGF_{2\alpha}$ of Ovsynch, CIDR and $PGF_{2\alpha}$ ovulation synchronization protocols, respectively, was similar ($P > 0.05$) (Table 3.1). Duration (days) of the ovulatory follicle was significantly ($P = 0.05$) higher in the Ovsynch than CIDR and $PGF_{2\alpha}$ protocols which were not different (Table 3.1). The diameter of the ovulatory follicle (mm) at the second injection was similar ($P > 0.05$) between Ovsynch and $PGF_{2\alpha}$ treatment protocols, but both were significantly larger ($P < 0.004$) than CIDR treatment protocol (Table 3.1).

3.5.2. LH profiles post synchronization and ovulation

3.5.2.1. The Ovsynch protocol

Figure 3.2a illustrates LH profiles of the Ovsynch treated heifers. A rise in LH surge was detected in four heifers at 2 h before the second GnRH injection. In one heifer the LH rise was detected just prior to administration of the second GnRH injection (0 h). On average, the LH peak occurred at 1.6 ± 0.9 h after the administration of the second GnRH injection. The peak ranged between 5-19 ng/ml and the area under the curve ranged between 13-62 units square. The duration (h) of LH surge ranged from 4 to 10 h (8 ± 2.4). Ovulation was between 22 to 24 h from LH peak. One heifer ovulated prematurely before the administration of the second GnRH injection and therefore was excluded from the statistical analysis.

3.5.2.2. The CIDR protocol

Figure 3.2b illustrates LH profiles of the CIDR treated heifers. The LH peak occurred around 19 ± 3.9 h after the second E_2 injection. The peak ranged between 9-23 ng/ml and the area under the curve ranged between 26-94 units square. The duration of LH surge lasted from 4 to 16 h (9.3 ± 4.3). Ovulation occurred between 24 to 38 h following the LH peak.

3.5.2.3. The $PGF_{2\alpha}$ protocol

Figure 3.2c illustrates LH profiles of the $PGF_{2\alpha}$ treated heifers. In five heifers, the LH peak occurred at 58 ± 4.4 h after the second $PGF_{2\alpha}$ injection. Peak concentration ranged from 3 to 9 ng/ml and the area under the curve ranged from 4 to 24 units square. The duration (h) of LH surge was between 2 and 14 h (7.2 ± 5.6). Ovulation occurred between 20 to 38 h from LH peak. One heifer ovulated prior to the set time for AI and thus was excluded from the statistical analysis.

The area under the curve for LH was significantly less ($P < 0.01$) in the $PGF_{2\alpha}$ heifers than Ovsynch and CIDR heifers, which were not different (Figure 3.3). The duration of LH surge (h) was not different ($P > 0.05$) between treatment protocols (Figure 3.4). The interval from the second injection of GnRH, E_2 or $PGF_{2\alpha}$ of the Ovsynch, CIDR and $PGF_{2\alpha}$ treatment protocols to ovulation was significantly different ($P < 0.03$) (Table 3.1). The interval from LH peak to ovulation was significantly shorter ($P < 0.05$) in the Ovsynch protocol relative to CIDR and $PGF_{2\alpha}$ treatment protocols, which were not different (Table 3.1).

3.5.3. Corpus luteum function post ovulation synchronization

Progesterone profiles from breeding to 14 or 22 days post AI are presented in Figures (3.5a-c) and (3.6a-c). For the Ovsynch treated heifers, P_4 started to rise above 1 ng/ml between 4 and 6 days after AI. It then continued to rise until day 10, remaining constant (6-7 ng/ml) between days 10-16. For the CIDR treated heifers, P_4 started to increase above 1 ng/ml around day 5 after AI. It continued to rise until day 10 when it remained constant (6-7 ng/ml) until day 16 after AI. Progesterone remained elevated (5.5 ng/ml) until day 22 post AI (last day of ultrasound) in one non-pregnant heifer whereas, in the other non-pregnant heifer P_4 decreased to < 1 ng/ml on day 18 post AI. For $PGF_{2\alpha}$ treated heifers, P_4 increased between days 4 and 6 after AI. It continued to rise until day 10, and remained constant until day 16 post AI. Functional luteal regression occurred between days 18 and 20 post AI in three heifers, whereas in one heifer it occurred on day 22 post AI.

The area under the curve for P_4 from breeding to 14 or 22 days post breeding was similar between treatment protocols ($P > 0.05$) (Figures 3.7 & 3.8). From day 8 post insemination until either day 14 or 22 post insemination, CL diameter was similar ($P > 0.05$) between Ovsynch, CIDR and $PGF_{2\alpha}$ treatment protocols. Progesterone area under the curve did not regress significantly on LH area under the curve. However, P_4 area under the curve regressed significantly ($P < 0.05$) and positively ($r = 0.4$) on LH surge duration.

3.5.4. Follicular dynamics post ovulation synchronization

3.5.4.1. The Ovsynch protocol

All heifers subjected to this protocol had two follicular wave cycles. Information on time of emergence of follicular waves, time of follicle deviation, duration of the dominance of the follicular waves, and maximum diameter of the 1st and 2nd wave DF, are all presented in Table 3.2. Follicular waves emerged on day 1 and 10 ± 0.0, for the first and second wave, respectively. The time of follicle deviation (when a DF is selected to be dominant) was on days 3 ± 0.0 and 13 ± 0.0 for the first and second wave, respectively. The duration of dominance of the first and second DF in the first and second waves, respectively, was 7 days. The maximum diameter of the first and second DF in the first and second follicular waves, respectively, averaged 18 ± 2.4 and 15 ± 0.72 mm.

3.5.4.2. The CIDR protocol

Five of six heifers had two follicular wave cycles, while the sixth heifer had three-wave cycle. Information on time of emergence of follicular waves, time of follicle deviation, duration of the dominance of the follicular waves, and maximum diameter of the 1st and 2nd wave DF, are all presented in Table 3.2. In the heifers with two follicular waves, the waves emerged on day 1 and 11.2 ± 1.8. Follicle deviation for the first and second follicular waves, respectively, was on days 3.5 ± 1.4 and 13.7 ± 2.3 of the estrous cycle. The duration of dominance of the first and second DF in the first and second follicular waves, respectively, was at 7 ± 1.6 and 5 ± 1.9 days. The maximum diameter of the first and second DF in the first and second follicular waves, respectively, was 14.8 ±

2.4 and 14.0 ± 0.65 mm. Follicular waves emerged on days 1, 10 and 17 in the heifer with three follicular waves and DF deviated on days 3, 12 and 19.

3.5.4.3. The $PGF_{2\alpha}$ protocol

Four out of five heifers had two follicular wave cycles, with the remaining heifer experiencing a three-wave cycle. Information on time of emergence of follicular waves, time of follicle deviation, duration of the dominance of the follicular waves, and maximum diameter of the 1st and 2nd wave DF, are all presented in Table 3.2. In the heifers with two follicular waves, wave emergence occurred on days 1 and 10.0 ± 1.6 for the first and second follicular waves, respectively. Dominant follicles deviated on days 3.0 ± 0.7 and 11.6 ± 1.7 of the estrous cycle for the first and second follicular waves, respectively. The duration of the dominance for the first and second follicular waves was 7.4 ± 0.55 and 6.8 ± 1.6 days, respectively. The maximum diameter of the first and second DF in the first and second follicular waves, respectively, was around 17.0 ± 1.1 and 16.8 ± 0.7 mm. The time of wave emergence in the heifer with three-wave cycle was on days 1, 8 and 15 of the estrous cycle. Deviation of the DF occurred on days 3, 9 and 16 of the estrous cycle.

While the time of the first follicular wave emergence was significantly different ($P < 0.0001$), the time of first wave deviation, duration of first wave DF and maximum diameter (mm) of the first wave DF were all not different ($P > 0.05$) between the treatment protocols (Table 3.2). Maximum diameter of the second wave DF was significantly larger ($P < 0.05$) in the $PGF_{2\alpha}$ treated animals than those in the Ovsynch and

CIDR treated groups which were not different ($P > 0.05$) (Table 3.2). However, the time of second wave emergence, second wave deviation, and duration of second wave DF were not different ($P > 0.05$) between the treatment protocols (Table 3.2). Ovulation synchronization rate was not different ($P > 0.05$) between treatment protocols, and PR was similar between Ovsynch and CIDR ($P > 0.05$) exposed heifers and both were significantly different ($P < 0.05$) from $PGF_{2\alpha}$ protocol group (Table 3.2).

3.6. Discussion

This study compared two newly developed protocols, Ovsynch and CIDR, that are aimed to synchronize ovulation, eliminate estrus detection and facilitate the use of timed AI in cattle. Pregnancy rates obtained in heifers following synchronization using Ovsynch protocol (Ambrose et al., 2000; Small et al., 2001) have been far less than those observed following breeding at natural estrus (Pursley et al., 1997a). However, PR in heifers following synchronization using the CIDR protocol have been comparable to those obtained when breeding immediately after natural estrus (Martinez et al., 2000; Martinez et al., 2002). Information on effects of these protocols on LH profile, CL function and follicular dynamics is limited. This is the first known study using dairy heifers in which a direct comparison was conducted between Ovsynch and CIDR ovulation synchronization/TAI protocols in terms of their effects on LH profiles, P_4 production and follicular dynamics, following synchronization.

3.6.1. Follicular dynamics during synchronization

The effectiveness of GnRH treatment is influenced by the stage of follicular development at the time of treatment (Pursley et al., 1995). Follicles in the growing phase (day 3.8) respond more to GnRH, thus ovulation, than follicles in the static (day 7.4) or regressing (day 12.7) phases (Silcox et al., 1993). Turnover of follicular waves in Ovsynch protocols is more rapid in heifers than lactating cows, increasing the probability of administering the first GnRH injection in the absence of a DF and/or turning over a follicular wave before ovulation can be induced with a second GnRH injection (Haughian and Wiltbank, 2002). The first GnRH injection of Ovsynch protocols does not always result in ovulation of the follicle. In this study, only one heifer ovulated as a result of the first GnRH injection which is in agreement with others (Pursley et al., 1995; Martinez et al., 1999; Castilho et al., 2000; Ambrose et al., 2001) who reported 30 to 54% ovulation rate in heifers after the first GnRH injection. Two heifers in this study, which did not respond to the first GnRH injection had low P_4 concentrations at the time of the $PGF_{2\alpha}$ injection. One of these two heifers ovulated before the second GnRH injection, in agreement with other studies, which reported 6% (Vasconcelos et al., 1999) to 10% (Stevenson et al., 1999) of cows ovulated before the second GnRH injection. The other heifer did not ovulate prior to the second GnRH injection, which is in agreement with Vasconcelos et al. (1999) who reported that 68% of the cows with low serum P_4 at the time of $PGF_{2\alpha}$ injection had a synchronized ovulation (ovulated after the second GnRH injection. Therefore, ovulation to the first GnRH injection is found to be necessary in the Ovsynch protocol, especially for cows in the late or early estrous cycle. The overall synchronization rate (cows that ovulated to second GnRH) varied depending on response

to first GnRH (92% if ovulation to first GnRH vs. 79% if no ovulation) (Vasconcelos et al., 1999). In this study, ovulation synchronization rate (after the second GnRH injection) in the heifers which did not ovulate after the first GnRH injection was at 80% (4/5).

In this study, E_2 and P_4 resulted in regression of DF in two heifers within 3 to 4 days, which is in agreement with other reports (Bo et al., 1995; Martinez et al., 1997; Garcia and Salaheddine, 2001). Estradiol-17β and P_4 suppress the growth of the follicle by prolonged suppression of FSH and LH, or by a direct effect on the follicle allowing for the emergence of a new follicular wave 3-5 days later (Rajamahendran and Manikkam, 1994; Twagiramungu et al., 1995). These heifers had high P_4 concentrations at the start of treatment, which is in agreement with Price and Webb (1988) and Bolt et al. (1990) who reported that estradiol suppressed gonadotropin secretion in the presence of high P_4. Four heifers subjected to the CIDR protocol were undergoing a new follicular wave at the start of the treatment and the resulting DF's at the time of the second E_2 injection were from the same follicular wave, which suggests that E_2 and P_4 did not affect gonadotropin secretion. The regression of the CL in dairy heifers emerges between days 16 and 19 of the estrous cycle (Ginther et al., 1989). Two heifers in this study were undergoing spontaneous CL regression when P_4 concentrations were halved at the time of $PGF_{2\alpha}$ injection, however, ovulation did not occur, probably because of P_4 in the CIDR device. Regardless of the stage of cycle of when CIDR protocol was initiated, ovulation synchronization rate was 100%.

In the present study, heifers in the $PGF_{2\alpha}$ protocol were at different stages of the cycle at the start of the first $PGF_{2\alpha}$ injection. For unknown reason, a single injection of $PGF_{2\alpha}$ does not cause luteolysis before day 5 of the estrous cycle (Lauderdale, 1972; Kiracofe et al., 1985). This was the case when one heifer was in early stage of the cycle and as a result did not undergo luteolysis as a response to the first $PGF_{2\alpha}$ injection. The rationale behind a two $PGF_{2\alpha}$ injections protocol was to overcome the circumstance of lack of response to $PGF_{2\alpha}$ when the animals are not at day 5 of the estrous cycle. The two injections of $PGF_{2\alpha}$ protocols are usually given 11 to 14 days, a part in heifers (Macmillan et al., 1978) and cows (Peters and Ball, 1987; Stephens and Rajamahendran, 1998). To achieve this recommended period (11 or 14 day intervals) animals must be between days 7 and 17 of the luteal phase of the estrous cycle (Nebel and Jobst, 1998). In this study and based on size of follicle and CL and concentration of P_4 at the time of first $PGF_{2\alpha}$ injection, the CL in heifers at the time of second $PGF_{2\alpha}$ injection were expected to be between days 5-9, 8-10, or 12-16 of the estrous cycle. Five of six heifers had synchronized ovulation.

Maintenance of the DF results in increased LH pulse frequency, which causes the DF to become persistent (Rajamahendran and Manikkam, 1994). Longer duration of dominance of preovulatory follicle has been found to have adverse effects on pregnancy success (Roche et al., 1999). Duration of dominance was longer in Ovsynch (7 days) than both CIDR (4 days) and $PGF_{2\alpha}$ (4 days) and all protocols yielded high ovulation synchronization rate and both CIDR and Ovsynch resulted in high PR. Duration of dominance of the ovulatory follicle in the animals exposed to the CIDR and Ovsynch

protocols and subsequent PR was similar to that found by Roche et al. (1999), and Moreira et al. (2000). Also, Moreira et al. (2000) reported that Ovsynch initiated on days 2 and 5 of the cycle resulted in more days of dominance (7 days) at the time of the second GnRH injection compared to days 10, 15, and 18 when treatment protocol was initiated.

The diameter of the DF at the time of the second injection was smaller in the heifers subjected to the CIDR treatment protocol than those treated with the Ovsynch and $PGF_{2\alpha}$ protocols. This is in contrast to the findings by Sanchez et al. (1995) who reported larger DF diameter as a result of using another form of P_4, norgestomet. The authors also reported that the norgestomet largest follicles were associated with higher E_2 concentrations. In cattle, pituitary gonadotrope cells are thought to be primed by E_2 during the estrous cycle (Reeves et al., 1971). Estradiol-17β is also responsible for stimulating LH surge (Beck and Convey, 1977). Accordingly, it was hypothesized that large follicles with increasing concentrations of E_2 could determine time of ovulation and magnitude of LH release (Lucy and Stevenson, 1986). In this study, E_2 was not measured, however, LH release was similar following Ovsynch and CIDR protocols, which confirms that functional dominance is more important than morphological dominance (Fortune et al., 1991). However, the largest diameter of the DF was attained at the time of the second injection of the respected ovulation synchronization protocols, which is in agreement with other work involving the Ovsynch protocol (Pursley et al., 1995; Cavestany et al., 2003).

Maximum diameter of DF at the second injection of treatment protocols did not influence subsequent P_4 concentration nor did it affect pregnancy. Progesterone area under the curve for the first 14 days and 22 days post AI was similar in the CIDR, Ovsynch and $PGF_{2\alpha}$ treatment protocols. Our results are in disagreement with Moreira et al. (2000) who found that larger follicles at the time of the second GnRH injection of Ovsynch protocol resulted in higher plasma P_4 concentrations. However, our results do agree with Moreira et al. (2000) with respect to the finding that the stage of the cycle when treatment protocols were initiated did not affect the size DF at the time of the second injection (stage by treatment was not examined in this study). Further, our results are in contrast with Vasconcelos et al. (1997) who reported lower PR as a result of larger DF's and that initiation of Ovsynch at metestrus, late diestrus or proestrus resulted in larger DF's at the time of second GnRH injection. Vasconcelos et al. (2001), however, observed that lactating dairy cows with small follicles had lower P_4 concentrations when compared to cows with large follicles. This was in contrast to the results of this study, in which smaller follicles in the CIDR protocols had similar P_4 levels for the first 14 days post AI compared to both Ovsynch and $PGF_{2\alpha}$ ovulation synchronization protocols.

3.6.2. LH profiles post ovulation synchronization

The main aim of ovulation synchronization protocols that are based on timed insemination is to control or advance the time of ovulation. Tight synchrony between timed AI and the time of ovulation enhances CL function and fertility. The inclusion of a second injection of E_2 following the removal of CIDR, or GnRH 48 h after $PGF_{2\alpha}$

(Ovsynch) has resulted in a more precise timing of estrus, LH and ovulation (Pursley et al., 1995; Hanlon et al., 1997; Lammoglia et al., 1998).

Estrus is accompanied by an LH surge, which occurs approximately 24 h following E_2 surge (Walters and Schallenberger, 1984) and peaks at the beginning of estrus (Hansel and Echternkamp, 1972). The LH areas under the curve in this study were similar between the CIDR and Ovsynch treatment protocols and both were higher than the $PGF_{2\alpha}$ treatment protocol's LH area under the curve. GnRH-induced LH occurs between 1 to 5 h (Johnson and Reeves, 1988; Rajamahendran et al., 1998), and remains elevated for up to 6 h (Johnson and Reeves, 1988; Rajamahendran et al., 1998). In this study, the duration of LH surge was similar between the treatment protocols. The premature LH surge (before second GnRH injection) in five heifers in the Ovsynch protocol suggests that these animals were at estrus prior to the second GnRH injection. Lucy and Stevenson (1986) found that GnRH-induced LH release after spontaneous LH surge in heifers was at 1.2 h, similar to that found in this study (1.6 h). These same authors reported that duration of LH surge ranged between 7-14 h, similar to LH surge duration observed in our study (8 h). Endogenous GnRH enhances endogenous LH and there is an additive effect between endogenous and exogenous GnRH, as stated by Lucy and Stevenson (1986) and as confirmed by the time of LH peak after the second GnRH injection observed in this study. The peak of GnRH responsiveness in the pituitary is around the time of estrus and prior to spontaneous LH surge (Kaltenback et al., 1974). Because blood sampling was conducted at 2 h intervals until time of ovulation, time of surge, peak and magnitude of LH may not have been very precise. The LH surge in

heifers is 10 h earlier than in cows (Lucy and Stevenson, 1986). However, premature LH surges in the Ovsynch heifers did not affect ovulation synchronization rate or PR. The time of ovulation following the second GnRH injection in the Ovsynch protocol (24 h) was within the range of that reported by Pursley et al. (1995).

In sheep, the ability of the hypothalamus to generate LH surge as a response of E_2 appears to be under the influence of P_4 treatment (Dobson et al., 1996). The action of P_4 is through the regulation of E_2 receptors at the site of ventromedial hypothalamus, where E_2 acts to induce LH surge (Blache et al., 1994). In cattle, E_2 stimulates a LH peak on a range between 18.5 to 25 h (Beck and Convey, 1977; Hanlon et al., 1997; Lammoglia et al., 1998) and this surge remains elevated for 8 hours (Beck and Convey, 1977; Hanlon et al., 1997). This is in agreement with our results in terms of LH peak (19 h) and duration of LH surge (9 h) after the second E_2 injection. All CIDR treated heifers ovulated after the second E_2 injection, which is in agreement with Hanlon et al. (1997), and in contrast with Hobson and Hansel (1972). None of the heifers in the CIDR protocol ovulated before the second E_2 injection. Exogenous as well as endogenous P_4 act to prevent E_2-induced LH surge in cattle with or without a functional CL (Rajamahendran et al., 1979; Bo et al., 1994). Low concentrations of P_4 block LH surge and ovulation (Rajamahendran and Manikkam, 1994).

A spontaneous or $PGF_{2\alpha}$-induced LH surge occurs between 50 and 60 h after $PGF_{2\alpha}$ (Walters and Schallenberger, 1984; Rajamahendran et al., 1989) and lasts between 6-10 h (Chenault et al., 1975 Walters and Schallenberger, 1984). Our results are in

agreement with these reports in terms of peak (53 h) and duration (7 h) of LH surge. Estrus and ovulation cannot be precisely synchronized with $PGF_{2\alpha}$ protocol because maturity of the DF and thus ovulation varies with individual heifers. Accordingly, this might have resulted in lower LH area under the curve and longer interval from the second injection of $PGF_{2\alpha}$ to ovulation, relative to other treatment protocols. A longer interval between the end of the synchronization treatment and the LH surge resulted in reduced transferable Grade 1 embryos (Lafri et al., 2002). Reduced embryo production as a result of delayed LH peak has been reported by other researchers (Donaldson, 1985; Goff et al., 1986). In this study, delayed LH peak following the second $PGF_{2\alpha}$ injection was associated with a concomitant reduction in maximal concentration of the preovulatory LH surge, in agreement with Lafri et al. (2002). The time of ovulation after the second $PGF_{2\alpha}$ injection in the $PGF_{2\alpha}$ protocol was similar to that observed by Rajamahendran et al. (1989).

3.6.3. Corpus luteum function post ovulation synchronization

Treatment protocols had no effect on CL structure and function. Progesterone concentrations were similar both during the first 14 days and throughout 22 days after AI. Similar profiles were found in both pregnant and open heifers in the same time period. All synchronized (responder) animals had $P_4 > 1$ ng/ml six days after AI. Progesterone profiles in this study are similar to those found by Moreira et al. (2000) who found similar P_4 concentrations from day of AI until 16 days later and that this was consistent regardless of when the Ovsynch protocol was initiated. A positive correlation has been found between P_4 concentration after insemination and PR (Butler et al., 1996), similar to

that found following use of the Ovsynch and CIDR protocols. A negative correlation was also found between post insemination P_4 and PR (Pritchard et al., 1994), similar to that found after $PGF_{2\alpha}$. Lucy and Stevenson (1986) found that heifers with GnRH-induced LH surges had lower subsequent P_4 concentration compared to control heifers, which was in contrast to our results. The authors proposed that GnRH effect on fertility might not be through enhancement of P_4 concentrations in treated animals. Other laboratories (Vasconcelos et al., 1999; Moreira et al., 2000) have stated that initiation of Ovsynch protocol at early or late stages of the cycle reduced rate of pregnancy. The pregnancy rate in this study (83%) is higher than the 40% PR following Ovsynch and fixed TAI in dairy heifers found by others (Schmitt et al., 1996; Pursley et al., 1997a,b; Stevenson et al., 2000). Pregnancy rates following CIDR protocol was at 67%, similar to that reported by others (Martinez et al., 2000; Martinez et al., 2002). Lucy et al. (1986) reported that PR following one fixed TAI at 80 h after the second of two injections of $PGF_{2\alpha}$ (23%) was less than that detected in similarly treated cows (51%) inseminated at estrus. This is in agreement with PR following $PGF_{2\alpha}$ found in this study. However, the number of heifers used in this study is small to make reliable comparisons on PR among treatment protocols.

3.6.4. Follicular dynamics post ovulation synchronization

Ultrasound imaging has revealed that follicular development occurred in two waves in normally cycling heifers and dairy cows (Pierson and Ginther, 1987; Burke et al., 1999). This was similar to the observations found in this study, in which 14/16 heifers had two wave cycles. However, Burke et al. (1999) observed that all dairy cows had three

wave cycles after treatment with estradiol benzoate and CIDR. Time of ovulation in this study was set as the day of wave emergence. Accordingly, time of first wave emergence was different between treatment protocols. Emergence of second follicular wave was on days 10, 11 and 10 for Ovsynch, CIDR and $PGF_{2\alpha}$ treatment protocols, respectively. Similar to our results, Moreira et al. (2000) found that emergence of second follicular wave occurred on day 11 or 12 after insemination for the days 2, 5, 10, 15 or 18 of the cycle when Ovsynch was initiated. Our results are also in agreement with Sirois and Fortune (1988) who reported that follicular waves started on days 2 and 11 in cows with 2 wave cycles. The time when the second DF deviated and duration of the second-wave DF did not differ between treatment protocols. However, for unknown reasons, the maximum size of the second-wave DF was larger in the $PGF_{2\alpha}$ protocol than Ovsynch and CIDR protocols. The mean day of the beginning the process of deviation between the two largest follicles within a follicular wave is day 2.8 (Ginther et al., 1996), similar to our findings.

3.7. Summary and conclusion

This was the first study in dairy heifers to directly compare the LH profiles, follicular dynamics, P_4 concentrations and PR following Ovsynch and CIDR ovulation and TAI synchronization protocols implemented at random stages of the estrous cycle. The study demonstrated that low ovulation rate to the first GnRH injection in the Ovsynch protocol did not influence ovulation synchronization rate following the second GnRH injection. However, initiation of Ovsynch protocol late in the estrous cycle appears to cause lower ovulation synchronization rates and PR, which is attributed to

premature ovulation. The study also illustrated that the diameter of the ovulatory DF, and duration of dominance of the ovulatory follicle, had no effect on subsequent LH profiles, follicular dynamics, P_4 concentration, or PR between synchronization protocols. Further, this study implies that occurring incidences of premature ovulation and thus lower PR in heifers following Ovsynch ovulation synchronization protocol may result from a premature LH surge prior to the second GnRH injection, which could adversely affect time of AI and subsequent fertility rate. The findings supported that use of the CIDR protocol consistently results in high ovulation synchronization as well PR in heifers, and comparable to those obtained when breeding immediately after natural estrus. In conclusion, similar post synchronization LH profiles, P_4 profiles and follicular dynamics following Ovsynch and CIDR ovulation synchronization protocols suggest that both protocols can be equally effective in synchronization of ovulation, elimination of estrus detection and enhancement of pregnancy in heifers.

Table 3.1. Effect of Ovsynch, CIDR, or $PGF_{2\alpha}$ ovulation synchronization/TAI protocols during synchronization on: 1) diameter of the largest follicle at the first injection of the treatment protocols; 2) duration of the ovulatory follicle (days) at time of the second injection of GnRH, E_2 or $PGF_{2\alpha}$ of the Ovsynch, CIDR or $PGF_{2\alpha}$ treatment protocols; 3) diameter of the largest follicle (mm) at the time of the second injection of GnRH, E_2 or $PGF_{2\alpha}$ of the Ovsynch, CIDR or $PGF_{2\alpha}$ treatment protocols; and 4) interval from LH peak to ovulation following treatment protocols. Different letters in each row represent significant difference. ($\alpha = 0.05$).

Parameters	Ovsynch	CIDR	PGF2α
Diameter of largest follicle (mean±SD) at 1st injection (mm)	12.3 ± 1.8 **a**	11.53 ± 2.0 **a**	12.6 ± 4.3 **a**
Duration of ovulatory follicle (d) (Mean±SE)	7.4 ± 1.14 **a**	4.0 ± 3.09 **b**	4.2 ± 1.78 **b**
Diameter of the ovulatory follicle (mean±SE) at 2nd injection (mm)	15.56 ± 0.63 **a**	12.0 ± 0.58 **b**	16.32 ± 0.63 **a**
Interval (mean±SE) from 2nd injection to ovulation (h)	24.0 ± 3.58 **a**	53.33 ± 3.27 **b**	88.8 ± 3.58 **c**
Interval (mean±SE) from LH peak to ovulation (h)	22.4 ± 2.55 **a**	30.0 ± 2.33 **b**	32.8 ± 2.55 **b**

[a] = $P > 0.05$

[b] = $P < 0.05$

c = $P < 0.05$

Table 3.2. Effect of Ovsynch, CIDR, or $PGF_{2\alpha}$ ovulation synchronization/TAI protocols on post synchronization follicular dynamics, ovulation synchronization rate, and pregnancy rates. Most heifers (14/16) had two follicular waves. The time of ovulation was considered the time of emergence of the first wave. Different letters in each row represent significant difference. ($\alpha = 0.05$).

Parameters	Ovsynch	CIDR	PGF2α
Time (mean±SD) of 1st wave DF deviation (d)	3.0 ± 0 a	3.5 ± 1.38 a	3.0 ± 0.71 a
Duration (mean±SD) of 1st wave DF (d)	7 ± 0 a	7.0 ± 1.26 a	7.4 ± 0.55 a
Diameter (mean ± SE) of 1st wave DF (mm)	18.06 ± 2.44 a	14.8 ± 2.38 a	17.04 ± 1.11 a
Time (mean ± SD) of 2nd wave emergence (d)	10 ± 0 a	11.17 ± 1.83 a	10.0 ± 1.58 a
Time (mean±SD) of 2nd wave deviation (d)	13 ± 0 a	13.67 ± 2.25 a	11.6 ± 1.67 a
Duration (mean±SD) of 2nd wave DF (d)	7 ± 0 a	5.17 ± 1.94 a	6.8 ± 1.64 a
Diameter (mean±SE) of 2nd wave DF (mm)	15.08 ± 0.72 a	14.05 ± 0.65 a	16.8 ± 0.72 b
Overall ovulation synchronization rate after the second injection	83% a	100% a	83% a
Overall pregnancy rates at Day 28 or 35 post AI	83% a	67% a	16% b

[a] = $P > 0.05$

[b] = $P < 0.05$

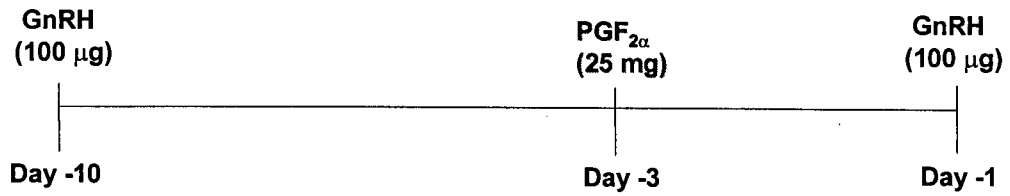

Figure 3.1a. The Ovsynch ovulation synchronization/TAI protocol which consisted of an initial injection of GnRH, followed 7 days later by an injection of $PGF_{2\alpha}$ and another GnRH injection 48 h later. Artificial insemination was conducted at 64 h after the $PGF_{2\alpha}$ injection.

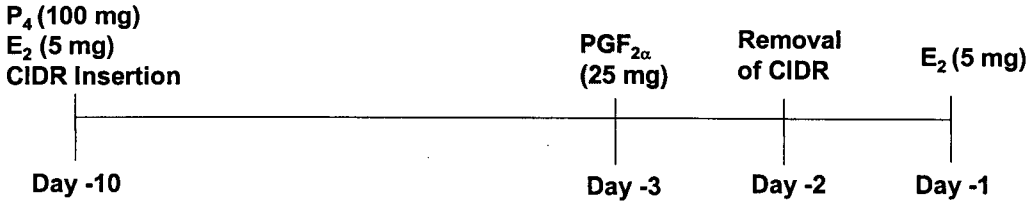

Figure 3.1b. The CIDR ovulation synchronization/TAI protocol which consisted of insertion of the CIDR device, an injection of progesterone and an initial injection of estradiol-17β on the first day of treatment, an injection of $PGF_{2\alpha}$ is administered 7 days later, followed by removal of CIDR 24 h later and another estradiol-17β injection 48 h after $PGF_{2\alpha}$. Artificial insemination was conducted at 76 h after the $PGF_{2\alpha}$ injection.

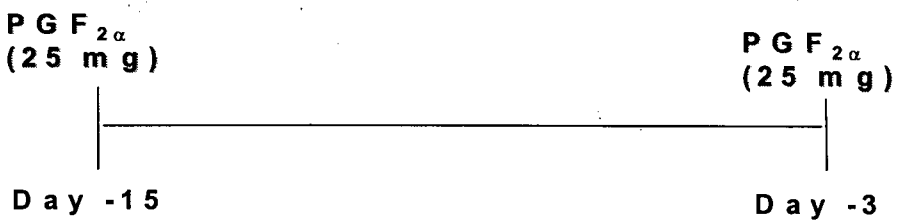

Figure 3.1c. Two $PGF_{2\alpha}$ injections ovulation synchronization/TAI protocol. The $PGF_{2\alpha}$ injections were administered 12 days apart. Artificial insemination was conducted at 72 h after the $PGF_{2\alpha}$ injection.

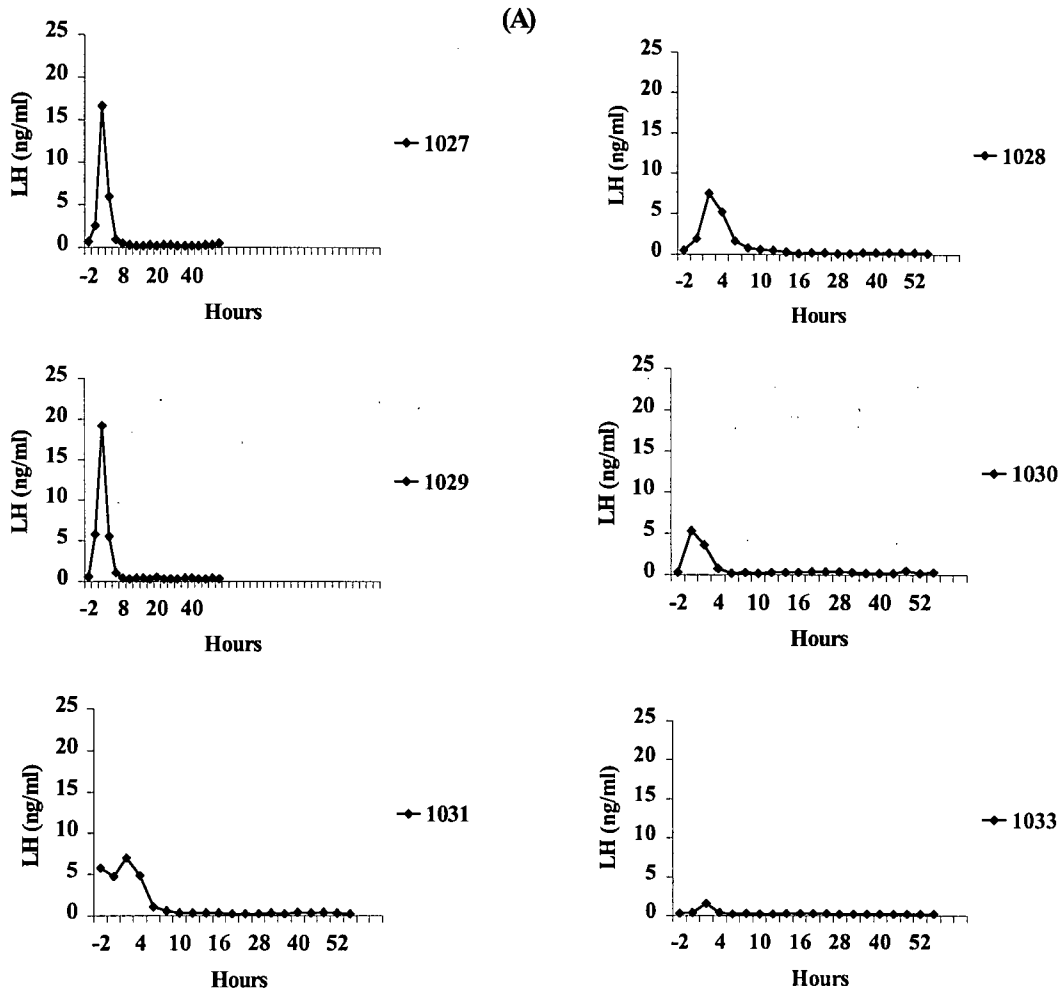

Figure 3.2a. LH profiles of individual heifers following treatment of Ovsynch synchronization protocol. Except for heifer# 1033 (which had premature ovulation) all five heifers had LH peak ranging between 5-19 ng/ml and area under the curve between 13-62 units square. The duration of LH surge ranged from 4 to 10h. Ovulation was between 22 to 24 h from LH peak.

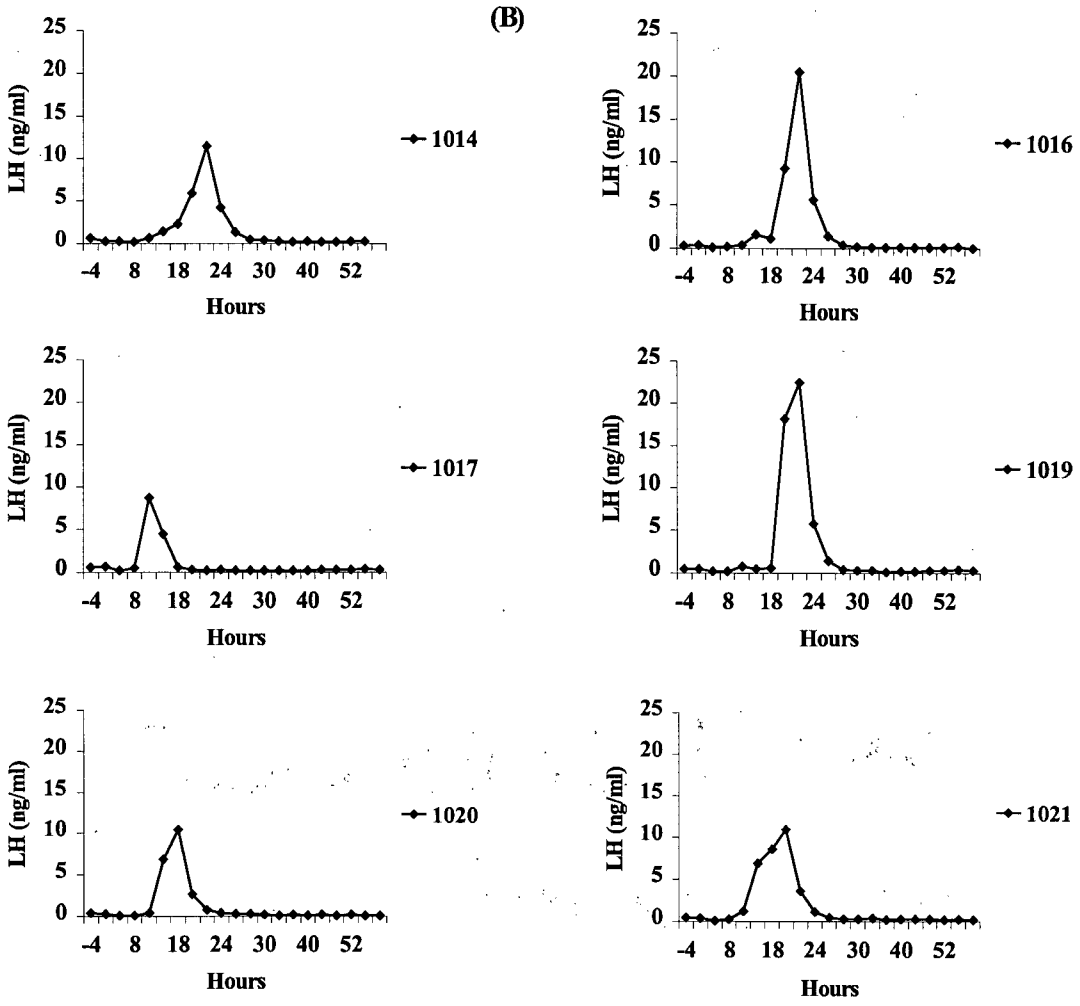

Figure 3.2b. LH profiles of individual heifers following treatment of CIDR ovulation synchronization protocol. The peak of LH ranged between 9-23 ng/ml and the area under the curve ranged between 26-94 units square. The duration of LH surge ranged from 4 to 16 h. Ovulation was between 24 to 38 h from LH peak.

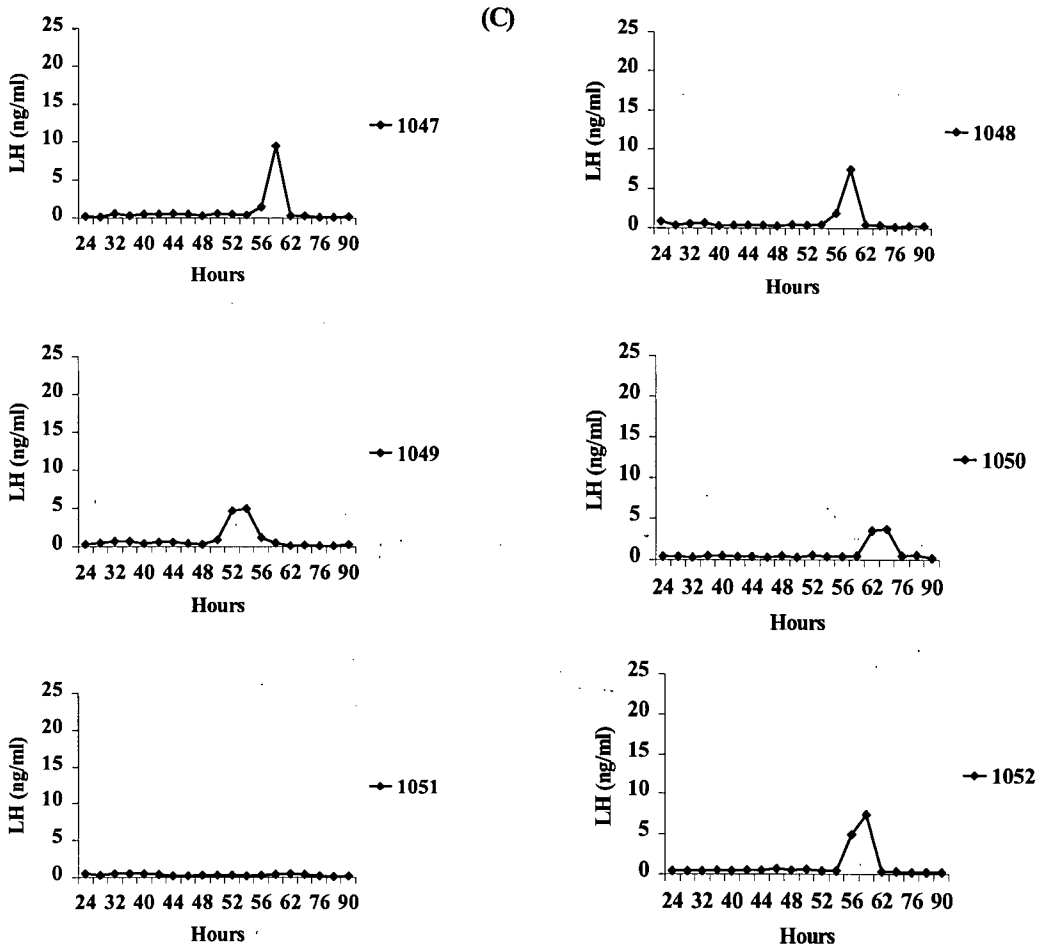

Figure 3.2c. LH profiles of individual heifers following treatment of $PGF_{2\alpha}$ ovulation synchronization protocol. Except for heifer#1051, all five heifers had LH peak from 3 to 9 ng/ml and the area under the curve was from 4 to 24 units square. The duration of LH surge was between 2 and 14 h. Ovulation was between 20 to 38 h from LH peak.

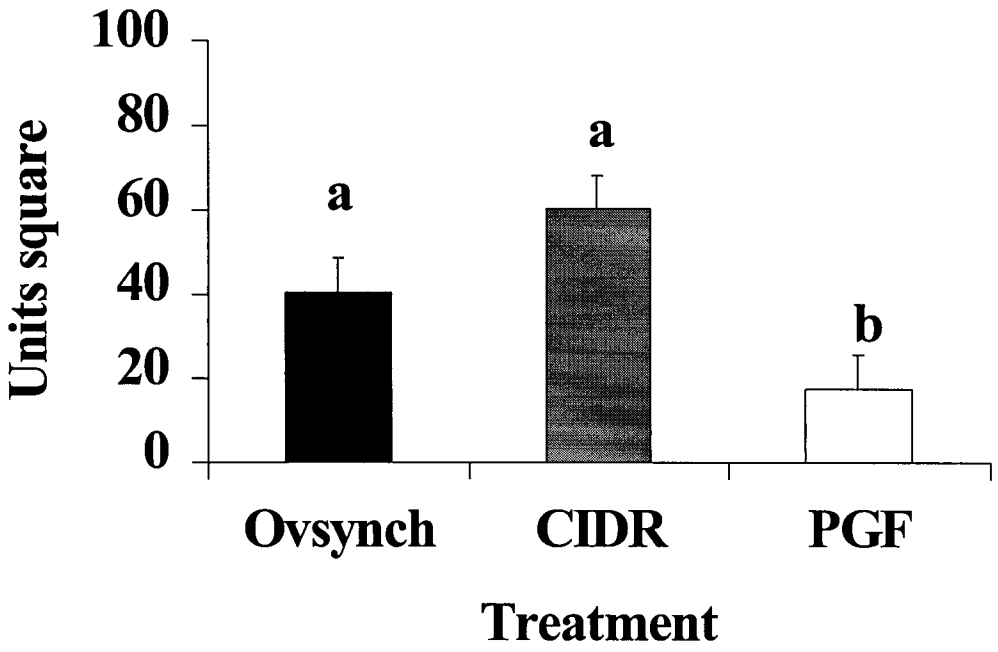

Figure 3.3. LH area under the curve of dairy heifers following treatment with Ovsynch, CIDR, or $PGF_{2\alpha}$ ovulation synchronization protocol. The area under the curve for LH (expressed in units square) was less ($P < 0.01$) in the $PGF_{2\alpha}$ heifers than Ovsynch and CIDR heifers, which were not different.

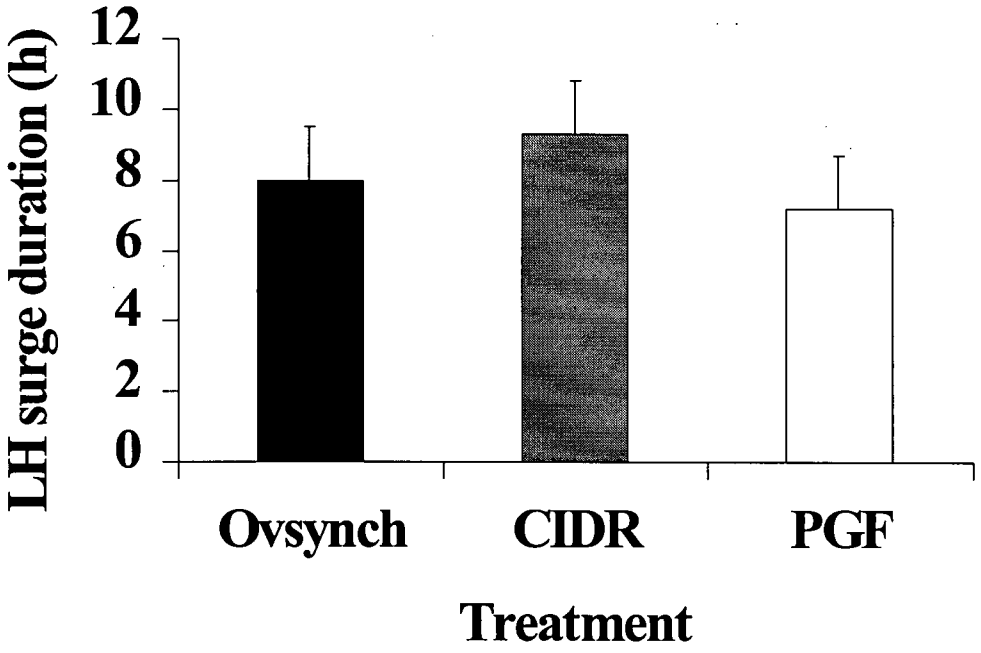

Figure 3.4. LH surge duration of dairy heifers following treatment with Ovsynch, CIDR, or $PGF_{2\alpha}$ ovulation synchronization protocol. The duration of LH surge (h) was not different ($P > 0.05$) between treatment protocols.

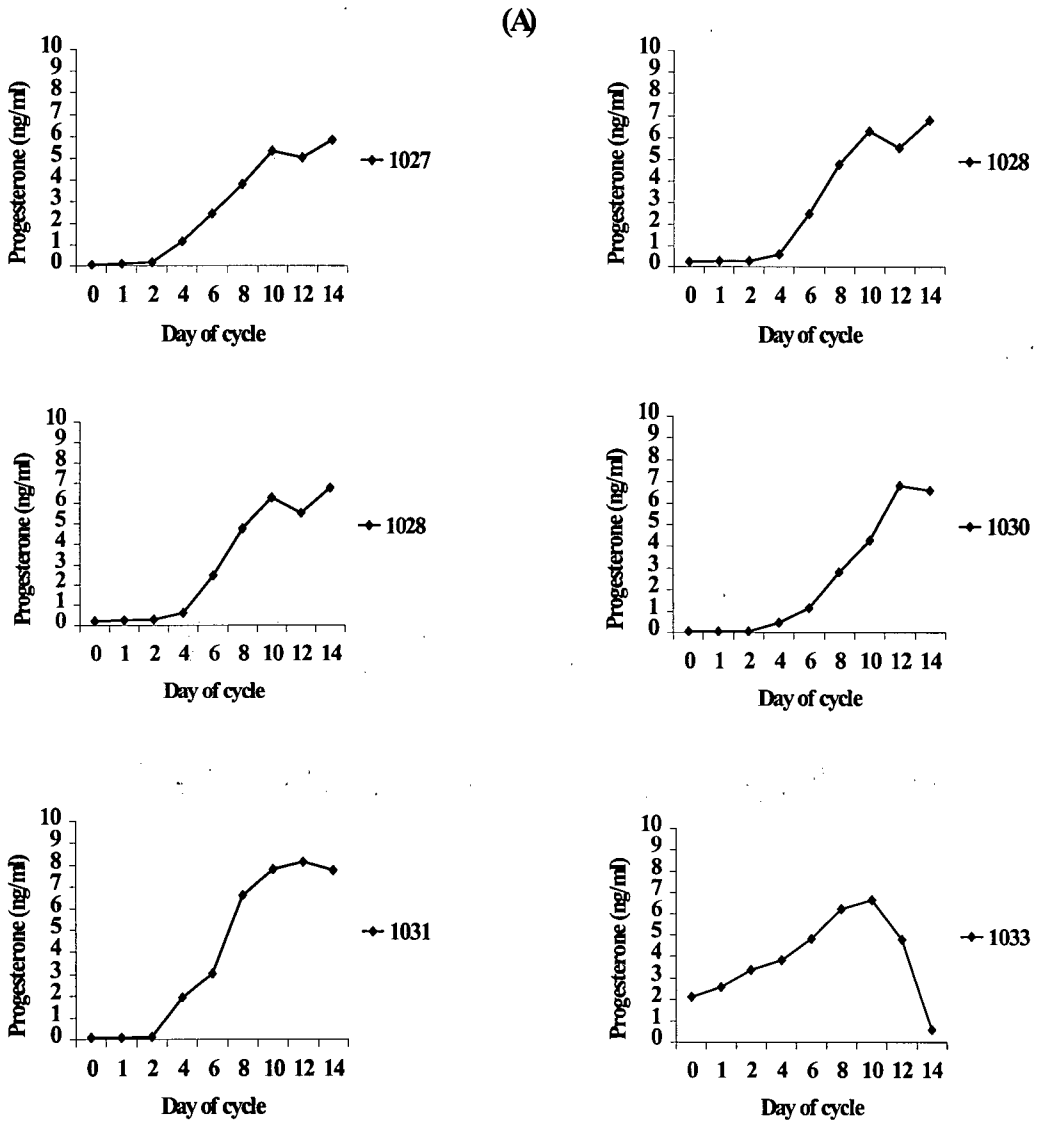

Figure 3.5a. Progesterone profiles of individual heifers from time of AI until 14 days post AI following treatment of Ovsynch protocol. Heifer#1033 ovulated before the second GnRH injection and as a result did not become pregnant. All other heifers were diagnosed pregnant by day 35 post AI.

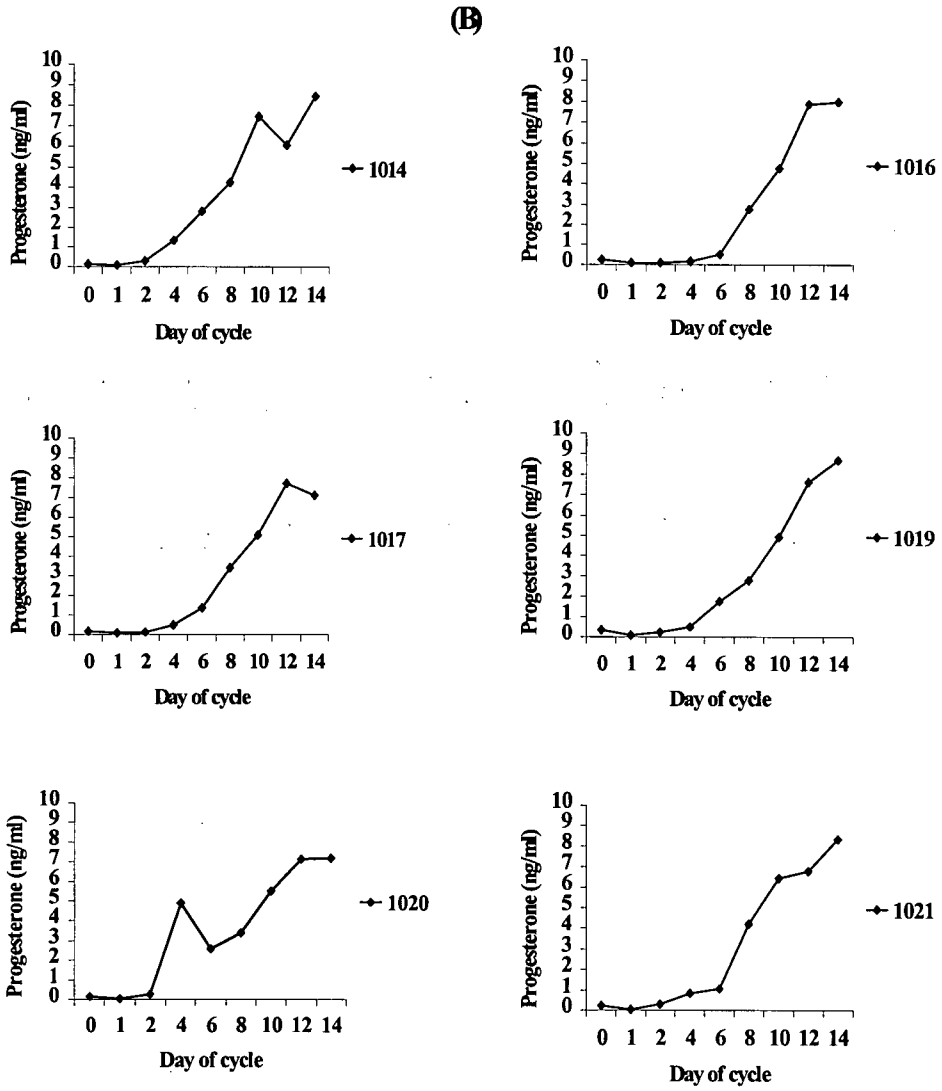

Figure 3.5b. Progesterone profiles of individual heifers from time of AI until 14 days post AI following treatment of CIDR protocol. Except for heifers#1020 & 1021, all other heifers were pregnant at day 35 post AI.

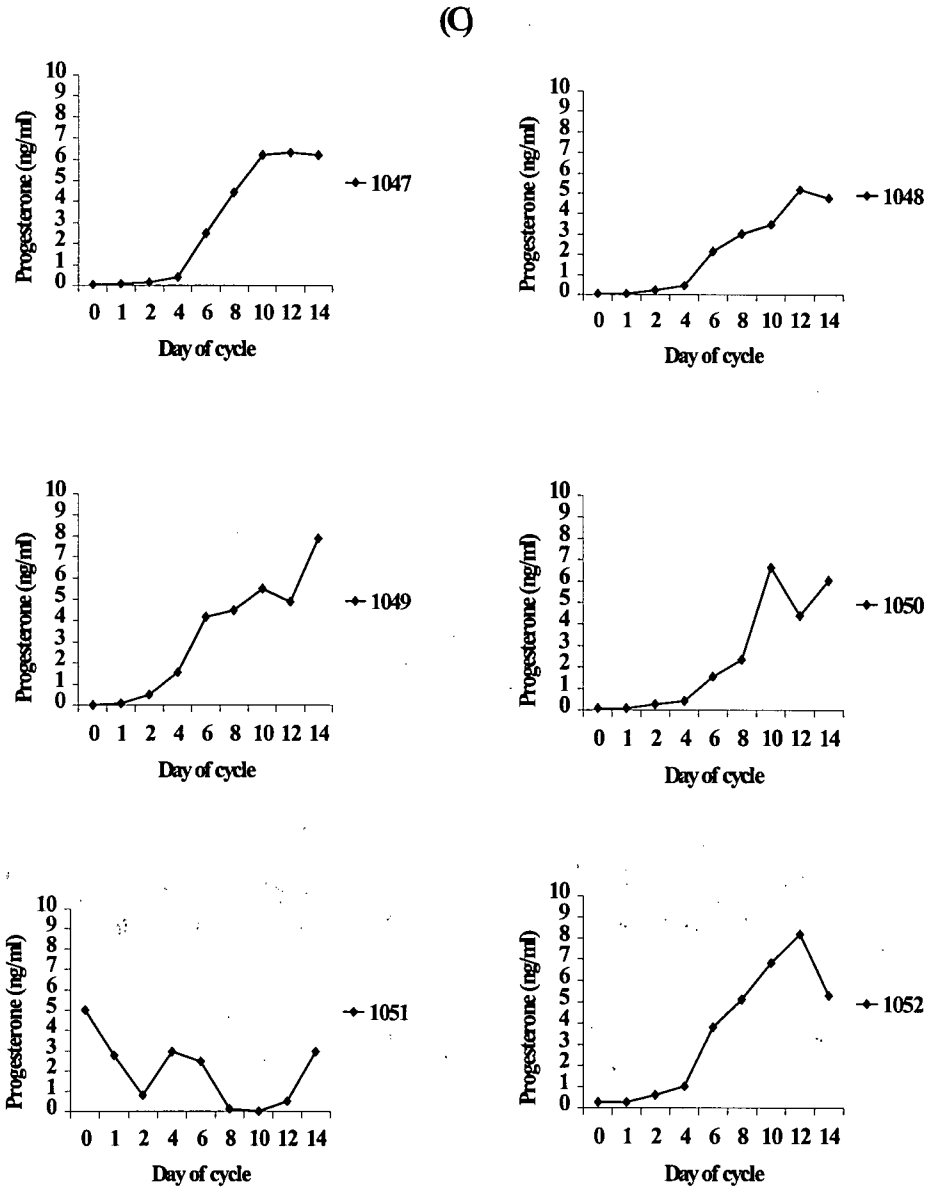

Figure 3.5c. Progesterone profiles of individual heifers from time of AI until 14 days post AI following treatment of $PGF_{2\alpha}$ protocol. Only heifer#1050 was pregnant at day 35 post AI.

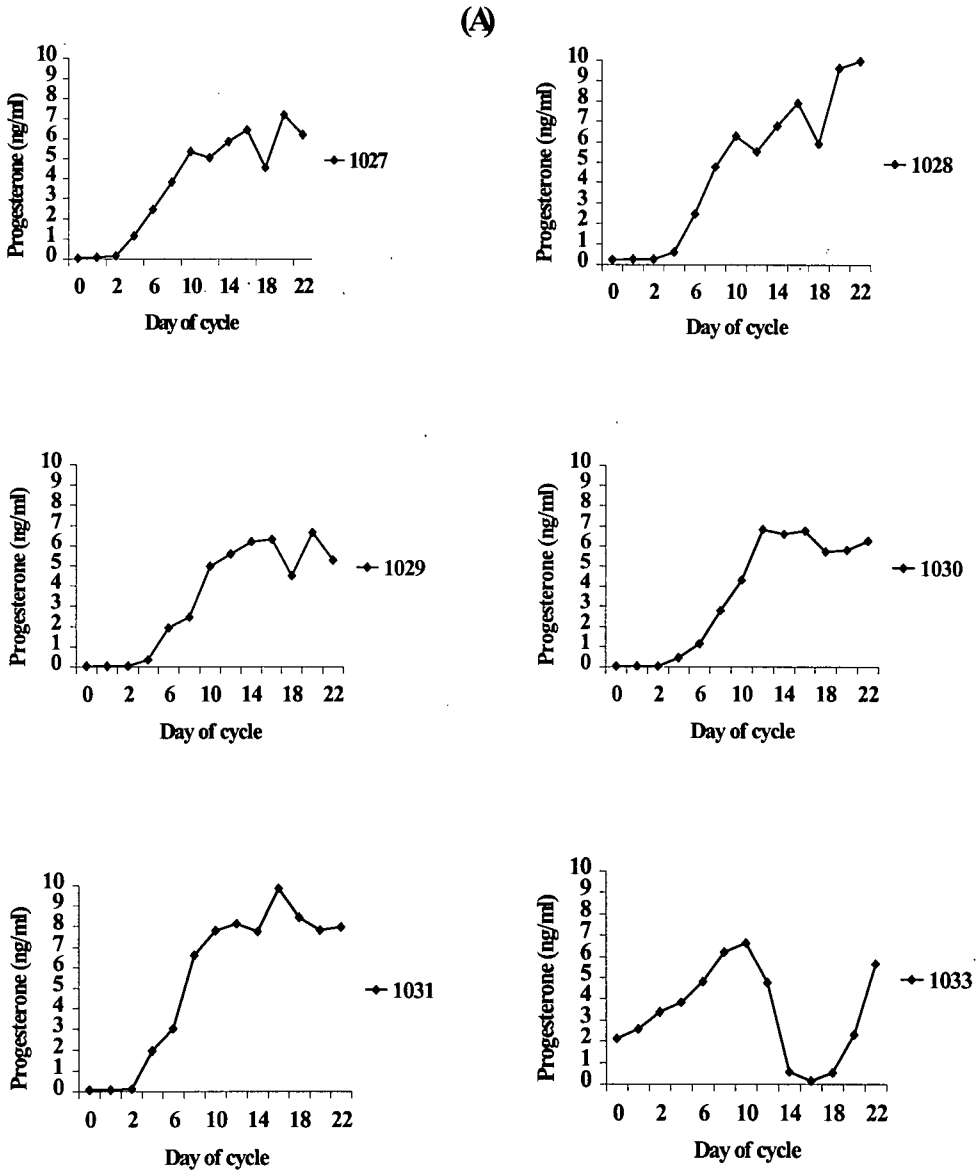

Figure 3.6a. Progesterone profiles of individual heifers from time of AI until 22 days post AI following treatment of Ovsynch protocol. Heifer#1033 ovulated before the second GnRH injection and as a result did not become pregnant. All other heifers were diagnosed pregnant by day 35 post AI.

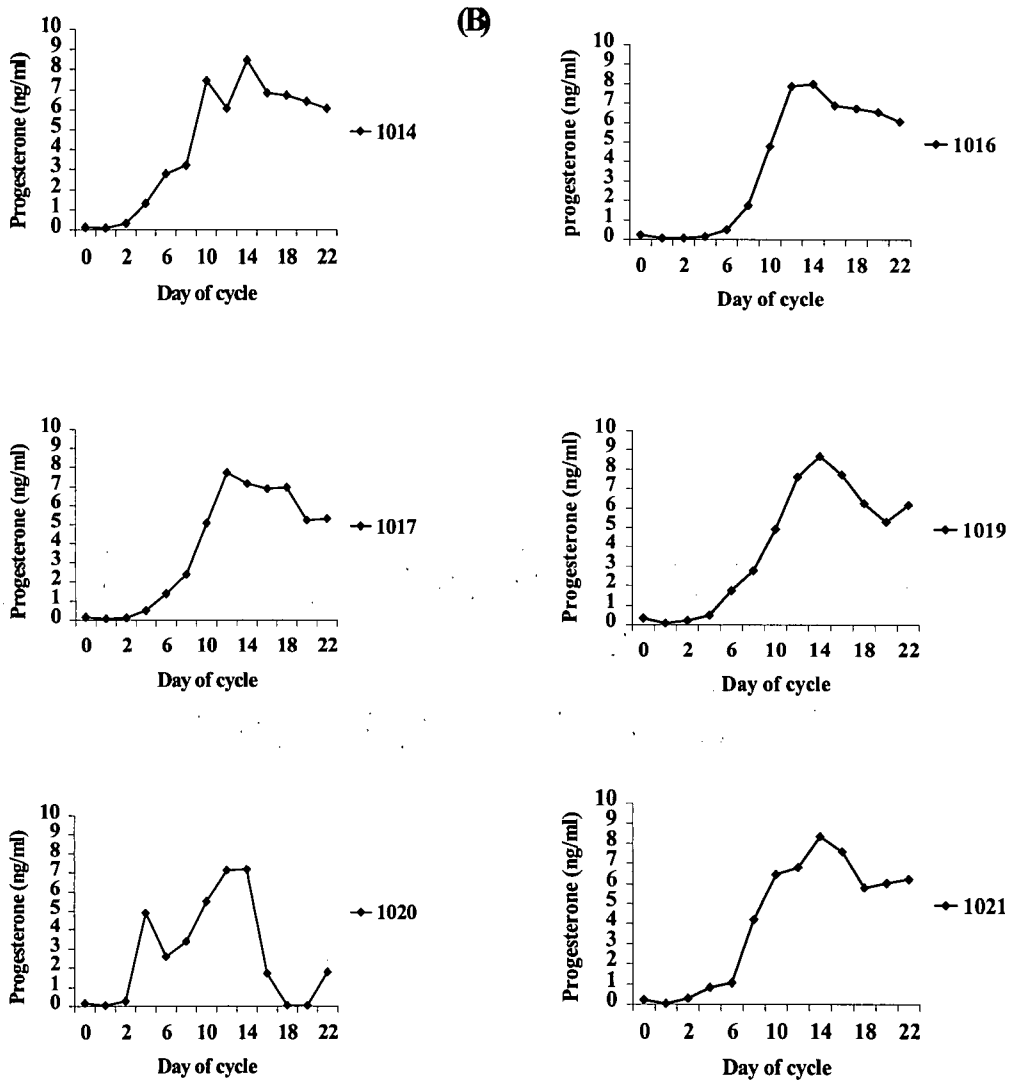

Figure 3.6b. Progesterone profiles of individual heifers from time of AI until 22 days post AI following treatment of CIDR protocol. Except for heifres#1020 & 1021, all other heifers were pregnant at day 35 post AI.

(C)

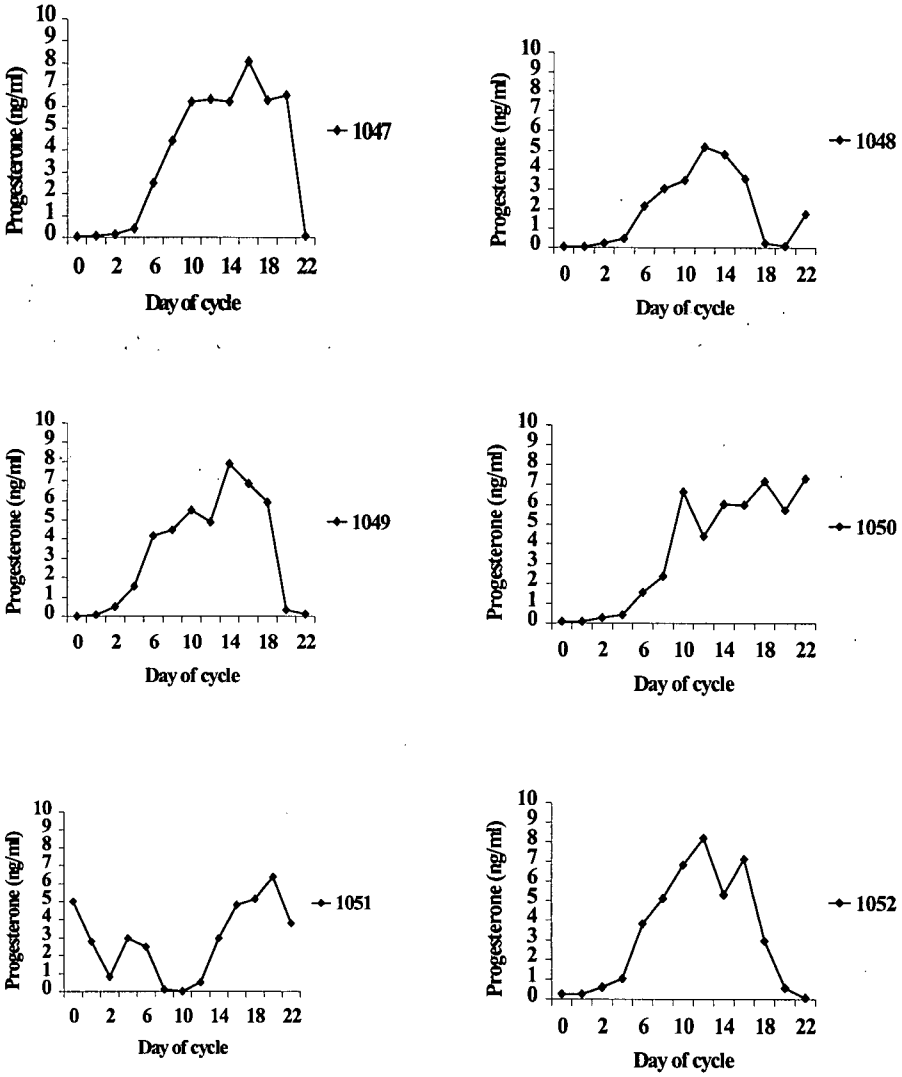

Figure 3.6c. Progesterone profiles of individual heifers from time of AI until 22 days post AI following treatment of $PGF_{2\alpha}$ protocol. Only heifer#1050 was pregnant at day 35 post AI.

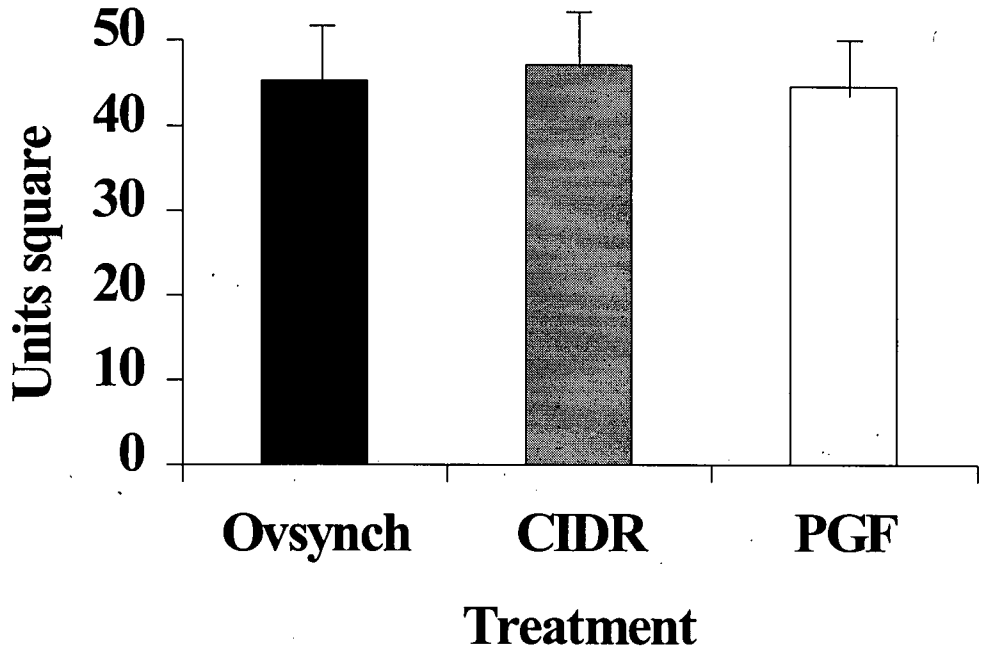

Figure 3.7. Progesterone area under the curve (first 14 days after AI) of dairy heifers following synchronization with Ovsynch, CIDR, or $PGF_{2\alpha}$ protocol. The area under the curve for P_4 from breeding to 14 days post breeding was similar between treatment protocols ($P > 0.05$).

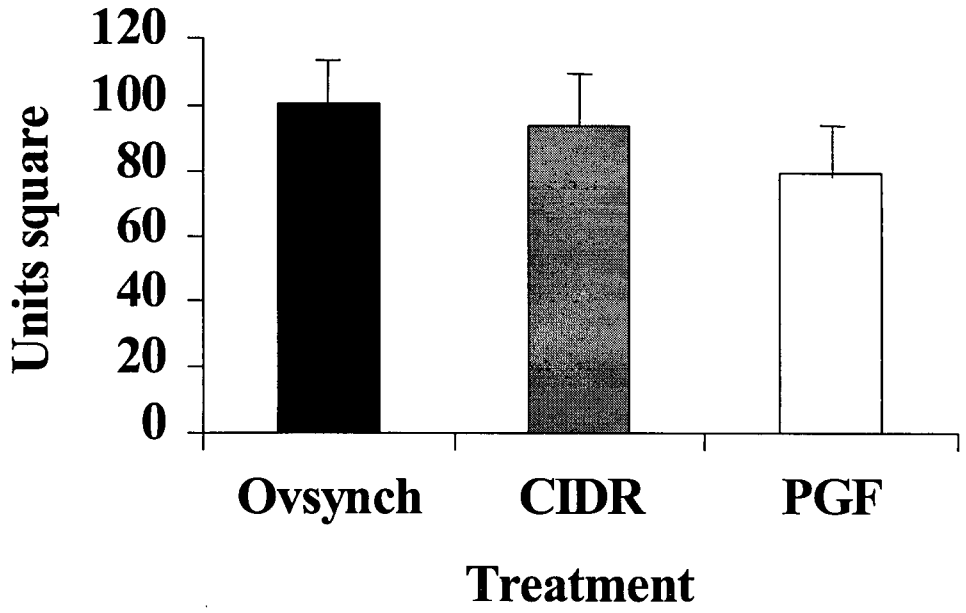

Figure 3.8. Progesterone area under the curve (for 22 days after AI) of dairy heifers following synchronization with Ovsynch, CIDR, or $PGF_{2\alpha}$ protocol. The area under the curve for P_4 from breeding to 22 days post breeding was similar between treatment protocols ($P > 0.05$).

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CHAPTER 4

IN VITRO ASSESSMENT OF CORPUS LUTEUM FUNCTION IN CATTLE, FOLLOWING OVSYNCH AND CIDR OVULATION SYNCHRONIZATION PROTOCOLS

4.1. Abstract

Non-lactating beef cows (n = 40) were used to determine in vitro production of progesterone (P_4) by corpora lutea (CL's) collected on days 6-8, 13-15 and 19-20 following synchronized ovulation of dominant follicles (DF's) that emerged after the Ovsynch or CIDR treatment protocols. The Ovsynch protocol consisted of an initial injection of gonadotropin-releasing hormone (GnRH; Factrel®; 100 μg), followed 7 days later by an injection of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; Lutalyse®) and 48 h later a second dose (100 μg) of GnRH (day = 0). The CIDR protocol consisted of initial injections of progesterone (P_4, 100 mg) and estradiol-17β (E_2, 5 mg) and insertion of controlled internal drug release (CIDR-B®, 1.9 g progesterone) followed 7 days later by an injection of $PGF_{2\alpha}$, 24 h later removal of CIDR-B®, and 48 h after $PGF_{2\alpha}$ a second 5 mg dose of E_2 (day = 0). Corpora lutea tissues were incubated (pre-incubation, without treatments, 1 h; with treatment, 6 h) with four different hormone treatments; LH, $PGF_{2\alpha}$, LH + $PGF_{2\alpha}$, and control (no treatment). In vitro P_4 did not differ ($P > 0.05$) between Ovsynch and CIDR but showed interaction ($P < 0.05$) between hormone treatment and stage of CL collection. For day 6-8, P_4 was similar between the control and $PGF_{2\alpha}$ and highest for LH and LH + $PGF_{2\alpha}$ treatments. For day 13-15, P_4 was lowest for the control, similar

between LH and $PGF_{2\alpha}$ and highest for LH + $PGF_{2\alpha}$. Progesterone was lowest for day 19-21 and did not differ ($P > 0.05$) among treatments. The CL formed after the Ovsynch and CIDR ovulation synchronization protocols yielded similar in vitro P_4 concentrations at different stages of the synchronized cycle.

4.2. Introduction

The significance of achieving a synchrony between the development of a highly functional ovulatory follicle as well as ovulation has been documented (Pursley et al., 1995; Hirad et al., 1999; Martinez et al., 2000; Small et al., 2001; Martinez et al., 2002; Cavestany et al., 2003). Ovsynch and CIDR are emerging protocols for ovulation synchronization that would facilitate artificial insemination of cattle at a fixed time (TAI). The Ovsynch treatment protocol uses an initial injection of gonadotropin releasing hormone (GnRH) to synchronize follicular wave emergence and induce or maintain a corpus luteum (CL) that is responsive to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) 7 days later. At 48 h after $PGF_{2\alpha}$, injection ovulation is synchronized by a second injection of GnRH (Pursley et al., 1995; Hirad et al., 1999; Peters and Pursley, 2003). The CIDR treatment protocol (Martinez et al., 2000; Ambrose et al., 2001) uses an initial injection of progesterone (P_4) and estradiol and an insertion of intravaginal P_4 device (CIDR-B®) to initiate a follicular wave and maintain P_4 until an injection of $PGF_{2\alpha}$ 7 days later. Then, the CIDR-B® device is removed and another estradiol injection is administered after $PGF_{2\alpha}$ injection to synchronize ovulation. (See chapter 2 for details on Ovsynch and CIDR ovulation synchronization protocols).

The CL is a product of ovulation of the ovulatory follicle, and a product of differentiation and proliferation of the follicular theca and granulosa cells (luteinization) during a transition period from a structure (ovulatory follicle) which mainly secretes estradiol to a structure, the CL, which mainly secretes P_4 (Niswender et al., 2000). In cattle, adequate CL, both structurally (maintenance of CL weight) and functionally (maintenance of P_4 production) throughout pregnancy is important for the maintenance of pregnancy and prevention of luteolysis. The growth and demise of the CL can be examined and followed by means of either rectal palpation or ultrasonography (Rajamahendran et al., 1994). The bovine CL increases in size until mid-luteal stage, and then decreases in diameter at late diestrus (Mares et al., 1962). In vivo P_4 secretion peaks around day 14 of the estrous cycle before it finally decreases three days before the onset of the next estrous cycle (Rajamahendran et al., 1976). The function of the CL can also be studied in vitro following CL enucleation and this means of study has been useful to study direct effects of stimulatory and inhibitory factors/hormones on synthesis of P_4 (Hansel et al., 1991). Armstrong and Black (1966) found that P_4 production per gram of luteal tissue did not differ until day 14 after estrus and concluded that it was the CL size, which determined P_4 production from early and mid-luteal CL. However, other laboratories (Rajamahendran et al., 1976; Watson and Munro, 1980; Grazul et al., 1989; Sprecher et al., 1989) found no correlation between CL size and CL function. Two mechanisms are involved in the control of bovine CL function; the first is the luteotropic mechanism responsible for the induction of the CL function, which involves luteinizing hormone (LH) (Simmons and Hansel, 1984); and the second mechanism is the luteolytic mechanism involved in the regression of the CL function, which involves $PGF_{2\alpha}$

(McCracken et al., 1973). Pregnancy rates (PR) have either been similar (Burke et al., 1996; Lammoglia et al., 1998) or higher for CIDR than Ovsynch (Martinez et al., 2000). Subluteal function has been shown to be associated with reduced fertility (Bulman and Lamming, 1978). Therefore, the objective of the present study was to compare in vitro CL P_4 production following Ovsynch and CIDR ovulation synchronization protocols.

4.3. Materials and Methods

4.3.1. Animals and treatments

The study was conducted at the Agriculture & Agri-Food Canada Research Centre, Brandon, Manitoba, Canada, using animals from the research centre herd. All handling and management of animals was in accordance with the guidelines of the Canadian Council on Animal Care (1993) and the Canadian Code of Practice for the care and handling of beef cattle (Agriculture Canada, 1990). Forty multiparous, non-lactating beef cows (60 to 90 days postpartum) were randomly assigned to either Ovsynch (n = 20) or CIDR (n = 20) synchronization protocols.

4.3.2. The Ovsynch protocol

The Ovsynch treatment protocol (Figure 4.1a) consisted of an initial injection of GnRH (100 μg i.m. of Factrel®; Fort Dodge Laboratories, Fort Dodge, IA), followed 7 days later by an injection of $PGF_{2\alpha}$ (25 mg i.m. of Dinoprost; Lutalyse®, Pharmacia & Upjohn, Orangeville, ON) and 48 h later by a second dose of 100 μg i.m. GnRH (day = 0).

4.3.3. The CIDR protocol

The CIDR treatment protocol (Figure 4.1b) consisted of initial injections of P_4 (100 mg; Sigma-Aldrich Canada Ltd) and estradiol-17β (E_2, 5 mg; Sigma-Aldrich Canada Ltd) and insertion of CIDR-B® (1.9 g progesterone; Inter-Ag, Hamilton, NZ) followed 7 days later by an injection of $PGF_{2\alpha}$, 24 h later removal of CIDR-B®, and 48 h after $PGF_{2\alpha}$ injection a second 5 mg dose of E_2 (day = 0).

4.3.4. Enucleation of the CL

Unilateral CL enucleation was carried out as previously described by Del Vecchio et al. (1995 a, b) at day 6 to 8, day 13 to 15, and day 19 to 21 after the second injection of either GnRH or E_2, for the Ovsynch and CIDR protocols, respectively. Since ovulation was expected to occur within 24 to 32 h after GnRH (Pursley et al., 1995) and within 39 to 64 h after estrogen (Bo et al., 1994; Hanlon et al., 1997; Lammoglia et al., 1998), these collection stages would represent CL collected during early-, mid- and late- luteal phases of the estrous cycle, respectively. Briefly, before each day of enucleation, six cows (three of each protocol) were rectally palpated to determine which ovary contained the CL. On the day of the surgery, the perineum of each cow was cleaned and prepared aseptically for surgery. Then, an epidural anaesthetic (5 ml of Lidocaine HCL 2%; MTC Pharmaceuticals, Cambridge, Ontario, Canada) was administered and the CL was removed via an incision in the anterior vagina with a medical grade scalpel. Enucleated CL's were placed in chilled and sterile phosphate-buffered saline (PBS; GIBCO BRL) containing gentamicin (50 μg/ml; Sigma-Aldrich Canada Ltd), before transfer within 20 minutes to the laboratory. The CL's were trimmed of exterior fat and connective tissue,

weighed, dissected into 50 mg pieces and distributed into 24-well plates that contained treatments.

4.3.5. Incubation of the CL

The CL tissues (50 mg) were incubated (pre-incubation, without treatment, 1 h; with treatments, 6 h) in serum-free medium (DMEM/HAM-F12; 1:1 v/v, Sigma-Aldrich Canada Ltd) + bovine serum albumen (BSA; 4 mg/ml; Fraction V, Sigma-Aldrich Canada Ltd), bovine insulin (12.5 μg/ml, Sigma-Aldrich Canada Ltd), transferrin (5 μg/ml); Sigma) and gentamicin (50 μg/ml, Sigma-Aldrich Canada Ltd). The treatments used were medium alone (control), media with bovine LH (USDA-LH-B5; Beltsville, MD), media with $PGF_{2\alpha}$ (Dinoprost; Lutalyse®; Pharmacia & Upjohn., Orangeville, ON), and media with LH and $PGF_{2\alpha}$. The concentrations for LH and $PGF_{2\alpha}$ were 500 ng per 50 mg/ml of medium-containing well. The plates without treatments were pre-incubated for 1 h at 37 °C, 5% CO_2 and 95% air. Following pre-incubation, the medium was replaced with fresh media which did or did not contain hormone treatments and was incubated for a further 6 h at 37 °C, 5% CO_2 and 95% air. At the end of incubation, the medium was collected and stored at -20 °C until radioimmunoassay of P_4. The concentrations of P_4 were expressed as per mg of total (50 mg) CL used during incubation period.

4.3.6. Ultrasonography, blood collection and P_4 analyses

Real-time transrectal ultrasonography (Aloka 500; 5.0 MHz probe, Aloka, Japan) was used to determine ovarian status on the day of $PGF_{2\alpha}$ injection (Small et al., 2001).

Blood was collected by coccygeal venipuncture into non-heparinized tubes on days -3, 0, and immediately before collection of CL. Blood was left at room temperature for at least 4 h, before centrifugation. Serum was aspirated and stored at -20 °C until analysis of P_4 using a commercially available solid-phase radioimmunoassay kit (Coat-A-Count; Diagnostic Products Corp., Los Angeles, CA). Aspirated medium was diluted 1:500 with phosphate buffered saline (10mM; pH 7.4; Sigma, St Louis, MO, USA) prior to P_4 assay as for blood serum. On the day of assay an aliquot of 100 µl of the standards was transferred into duplicate appropriate antibody coated tubes labeled A, B, C, D, E, F, and G. The standard tubes (A through G) corresponded to P_4 concentrations of 0, 0.1, 0.5, 2.0, 10.0, 20.0, and 40.0 ng/ml. Then, P_4-buffered I^{125} -labeled P_4 (1.0 ml) was added to all tubes. The tubes were then mixed and incubated at room temperature for 3 h for equilibrium. After incubation, the tubes were decanted and counted for 1 min using the gamma counter (Packard Auto gamma 500, Packard Instruments, Downers Grove, IL, USA). The sensitivity, intra- and inter- assay coefficient of variation were 0.01 ng/ml, 7.2% and 7.5% (n=3), respectively.

4.4. Statistical analysis

Least squared analysis of co-variance using the JMP IN® statistical package (2001) was used. The main effects were synchronization treatment, stages of the estrous cycle, and hormone effect in culture. Two-way interaction (synchronization x stage; synchronization x hormone) and three-way interaction (synchronization x stage x hormone) were examined. CL weight in culture was used as a covariate. The level of significance was set at $\alpha = 0.05$.

4.5. Results

4.5.1. Ultrasound and P_4 data

Seven out of the 20 Ovsynch-treated cows had follicles between 8 and 18 mm in diameter and regressing CL and these animals had P_4 < 1.0 ng/ml on the day of $PGF_{2\alpha}$ injection (day -3). Two of these cows showed clear signs of estrus (vaginal mucus) before the second injection of GnRH (day 0), while the other five cows had large follicles but did not show signs of estrus. The remainder of the Ovsynch-treated cows had one large CL and P_4 > 1 ng/ml on day -3. However, all Ovsynch-treated cows had P_4 < 1.0 ng/ml on day 0, and all the cows during the early stages of the cycle had CL weight between 1.6 to 6.0 g and P_4 concentrations between 1.5 and 12.0 ng/ml. Five out of the 20 CIDR-treated cows had large follicles (13-21mm in diameter), no CL and P_4 of < 1.0 ng/ml at the time of $PGF_{2\alpha}$ injection (day -3). These cows did not show estrus prior to the second injection of E_2 (day 0). The remainder of the CIDR-treated cows had large CL at the time of the $PGF_{2\alpha}$ and serum P_4 was greater than 1.0 ng/ml for all cows with large CL at this time. Progesterone concentrations were less than 1.0 ng/ml on day 0 for all CIDR-treated cows, and all the cows during the early stages of the cycle had CL weight between 1.7 and 5.3 g and P_4 concentrations between 1.4 and 2.3 ng/ml.

4.5.2. Post treatment <u>in vitro</u> P_4 production

Enucleation of CL was carried out in 33 cows, 10 early luteal (Ovsynch n = 5; CIDR n = 5), 12 mid-luteal (Ovsynch n = 6; CIDR n = 6) and 11 late-luteal (Ovsynch n = 6; CIDR n = 5). Seven cows were excluded from the study; two CL's from two animals (one from each treatment) were lost in the body cavity; two animals (one from each

treatment) had no distinctive CL on the day of surgery (either day 14 or 15 of CL collection), which indicate that these cows had short luteal phase; however, one of the cows (Ovsynch) had P_4 levels at 2.6 ng/ml; and the other three cows had problems such as vaginal infection, prolapsed uterus and cystic ovary.

Figure 4.2 shows P_4 production by CL's collected from beef cows at early-, mid- and late- luteal phases of the estrous cycle following Ovsynch and the CIDR ovulation synchronization protocols, and subjected to LH, $PGF_{2\alpha}$, and LH + $PGF_{2\alpha}$ treatments in virto. In vitro P_4 production following incubation with medium alone (control), LH, $PGF_{2\alpha}$, and LH + $PGF_{2\alpha}$ did not differ ($P > 0.05$) between CL's collected at early-, mid-, and late- luteal phases of the estrous cycle. Therefore, data for the two synchronization protocols were combined and the effects of media alone (control), LH, $PGF_{2\alpha}$, and LH + $PGF_{2\alpha}$ were further analyzed. The data showed interaction ($P < 0.05$) between in vitro hormone treatments and stage of CL collection (Figure 4.3). In vitro P_4 production during the 1 h pre-incubation period was not different ($P > 0.05$) between prospective LH, $PGF_{2\alpha}$, and LH + $PGF_{2\alpha}$ treatment wells (data not shown). Progesterone for the untreated control at 6 h was greater than 1 h only for day 6-8 and at this time P_4 was similar between the control and $PGF_{2\alpha}$ and highest for LH and LH + $PGF_{2\alpha}$ treatments (Figure 4.3). For day 13-15, P_4 was lowest for the control, similar between LH and $PGF_{2\alpha}$ and highest for LH + $PGF_{2\alpha}$ (Figure 4.3). Progesterone was lowest for day 19-21 and did not differ ($P > 0.05$) among treatment protocols (Figure 4.3).

4.5.3. Post treatment serum P_4 production

There was no significant interaction ($P > 0.05$) between the effect of synchronization treatment and stage of the estrous cycle in terms of serum P_4 concentrations. Serum P_4 was lowest for day 19-21 and similar between day 6-8 and day 13-15 (1.0, 3.7 and 4.7 ± 1.1 ng/ml, respectively $P > 0.05$). Total CL weight was highest for day 13-15 and similar between day 6-8 and day 19-21 (4.7, 3.7 and 2.3 ± 0.45 g, respectively; $P > 0.05$).

4.6. Discussion

This study has demonstrated that in vitro P_4 production by CL is similar following Ovsynch and CIDR based ovulation synchronization protocols. Some studies have shown that a CIDR based protocol results in higher PR (Martinez et al., 2000) than protocols like Ovsynch (Burke et al., 1996) that do not use a progestin. Others reported no difference between CIDR- based treatment (Lammoglia et al., 1998) and Ovsynch based treatment (Burke et al., 1996). This study suggests that any differences in fertility between the two protocols cannot be explained by treatment effect on CL function.

4.6.1. Effect of treatment protocol on synchronization

The ovulation synchronization rate to the initial injection of GnRH was in agreement with other reported ovulation synchronization rates (Pursley et al. 1995, 1997; Martinez et al. 1999; Vasconcelos et al., 1999; Moreira et al., 2000; Cavestany et al., 2003). Martinez et al. (1999) reported that the first GnRH induced a new follicular wave only when ovulation occurred in response to treatment and ovulation after the first GnRH injection resulted in a better synchrony, mainly when the Ovsynch protocol was initiated

between days 5 to 9 of the estrous cycle (Vasconcelos et al., 1999). The reported signs of estrus were recorded when the animals were scanned on day -3 of the synchronization protocols. Among the Ovsynch cows, two out of 17 showed clear signs of estrus (vaginal mucus) on the day of $PGF_{2\alpha}$ injection, 48 h before the second injection of GnRH. The P_4 levels of these cows on day -3 were < 1 ng/ml and the follicle diameter were 16 and 18 mm. Five out of 17 cows of the Ovsynch protocol had follicle diameters between 8 and 17 mm, and their P_4 levels were < 1 ng/ml. However, Henricks (1971) reported that most of standing heat incidences occurred at night, and therefore, the animals with P_4 < 1.0 ng/ml and large follicle diameter may have expressed signs of estrus and could not be detected. Cows with DF of small diameter had longer proestrus period in comparison with cows, which had larger DF (Sirois and Fortune, 1988). Follicles of 10 mm in diameter required a greater LH dose to induce ovulation compared with larger follicles (Fortune et al., 1991). Acquisition of LH receptors on the granulosa cells, a characterizing factor of follicle dominance does not occur before follicle deviation (day 2.8), when the diameter of the DF and subordinate follicles are 8.5 mm and 7.2 mm, respectively (Xu et al., 1995, Bodensteiner et al., 1996). However, in a more recent study, Beg et al. (2001) reported that the DF acquires LH receptor at a diameter of 8 mm. Several studies have reported incidences of premature estrus as well as ovulation before the second GnRH injection of the Ovsynch protocol, in cows as well as heifers with P_4 < 1 ng/ml at the time of $PGF_{2\alpha}$ injection (Pursley et al., 1995, 1997; Martinez et al.1999; Vasconcelos et al., 1999; Moreira et al., 2000; Cavestany et al., 2003). In contrast, Vasconcelos et al. (1999) reported that 68% of the cows, which had low serum P_4 at the time of $PGF_{2\alpha}$ had a synchronized ovulation (ovulated after the second GnRH injection).

This suggests that the early stage (day 6-8) CL formed after Ovsynch treatment were newly formed CL. Most of the cows (10/17) given the Ovsynch had large CL and high P_4 levels on day -3 when $PGF_{2\alpha}$ was injected.

Consistent results have been reported in terms of DF elimination and follicular wave initiation, when estrogens were given in combination with P_4 regardless of the stage of the estrous cycle (Bo et al., 1994; Garcia and Salaheddine, 2001). Researchers utilizing CIDR-B® devices in ovulation synchronization protocols have reported PR in beef heifers, beef cows, and dairy cows ranging from 30% to 80% (Lammoglia et al., 1998; Martinez et al., 2000; Day et al., 2000; Ambrose et al., 2001). At the time of CIDR-B® insertion the cattle were usually injected with estrogen and P_4 to synchronize emergence of a new wave of follicles, $PGF_{2\alpha}$ was given 7 days later when the device was removed, and estrogen was injected 24 h later to synchronize ovulation for TAI. Five of the CIDR animals in this study had large follicles and no CL at the time of $PGF_{2\alpha}$ injection. These large follicles could have been the result of a new follicular wave. None of the five cows had visible signs of estrus at the $PGF_{2\alpha}$ injection. This could have been due to the effects of the P_4 released from the CIDR device. Hobson and Hansel (1972) stated that P_4 prevented LH stimulation by E_2. The remaining cows of the CIDR protocol had large CL at the time of the $PGF_{2\alpha}$ injection. These CL may have been the result of ovulating DF's prior to the start of the protocol. All the CL were functional based on the concentrations of the P_4 in the serum on the day of $PGF_{2\alpha}$ administration.

4.6.2. Post treatment <u>in vitro</u> P_4 production

This study showed that P_4 production by the early luteal phase CL was stimulated only by LH and LH + $PGF_{2\alpha}$ whereas LH, $PGF_{2\alpha}$ and LH + $PGF_{2\alpha}$ had additive effects on P_4 production by mid- luteal phase CL, and late luteal phase CL were unresponsive to LH, $PGF_{2\alpha}$ or LH + $PGF_{2\alpha}$. The in vitro conditions were similar to those of Redmer et al. (1988), and the time of incubation (6 h) was comparable to that of others (Armstrong and Black, 1966; Redmer et al., 1988); conditions which also showed that in vitro P_4 production was higher at early- and mid- than late- luteal phase and stimulated by LH.

Organ culture preserves cell interaction and retains histological and biochemical differentiation of the CL. More P_4 was produced as a result of LH, when both the small and large luteal cells were mixed rather than separated (Harrison et al., 1987; Del Vecchio et al., 1995a, b). The support of LH is necessary for luteal development and P_4 production from early- until mid- luteal phases of the estrous cycle (Peters et al., 1994). In cattle, both small and large luteal cells have receptors for LH (Niswender et al., 2000). It was proposed that LH binds its receptors in small luteal cells and stimulates protein kinase A activity and an increase in P_4 (Harrison et al., 1987; Hansel et al., 1991; Niswender et al., 2000). Throughout the estrous cycle, only 2% of LH total receptors are occupied (Garverick et al., 1986). The same authors found a significant and positive correlation between high P_4 in circulation and occupied LH receptors between days 4-10 of the estrous cycle. In addition, only the occupied receptors get to activate G-protein, adenylate cyclase enzyme, and cAMP second messenger system (Stryer, 1988). The

results of this study imply that more LH receptors were occupied during early and mid-luteal phases of the estrous cycle.

The large luteal cells have been shown to possess receptors for prostaglandin-I_2, prostaglandin-E_2 and $PGF_{2\alpha}$ (Niswender et al., 2000). Prostaglandins E_2 and I_2 have luteotropic properties, whereas $PGF_{2\alpha}$ is mainly luteolytic (Niswender et al., 2000). However, $PGF_{2\alpha}$ has also been shown to stimulate P_4 secretion in vitro (Speroff and Ramwell, 1970; Redmer et al., 1988; Hansel et al., 1991). Hansel et al. (1991) stated that the stimulatory effect occurs in small luteal cells via phospholipase C mechanism. Our results were in concert with the results of these scientists, when $PGF_{2\alpha}$ stimulated P_4 production in mid- luteal phase CL and in disagreement with Hall and Robinson (1979) who used the same dosage (500 μg) and showed inhibitory effects on rat luteal tissue with respect to P_4 synthesis. In large luteal cells, arachidonic acid stimulated $PGF_{2\alpha}$, which inhibits P_4 secretion, but in small luteal cells, arachidonic acid stimulated production of prostaglandins I_2 and E_2 or stimulated the uptake of cholesterol and the increase in P_4 production (Del Vecchio et al., 1995a). In NIH 3T3 cells, there is evidence for two forms of the $PGF_{2\alpha}$ receptors, one that couples to adenylate cyclase and does not exhibit selectivity between prostaglandins-$F_{2\alpha}$ and E_2 and another which couples to phospholipase C and shows a 40-fold selectivity towards $PGF_{2\alpha}$ over prostaglandinE_2 (Gusovsky, 1991). Wiltbank et al. (1995) stated that no significant difference between developing (days 2 and 4) and active (days 6 and 10) bovine CL in either affinity or concentration of $PGF_{2\alpha}$ receptors. This suggests that the in vitro action of $PGF_{2\alpha}$ in this study is not receptor dependent.

The combined treatment of LH + $PGF_{2\alpha}$ had a significant effect on P_4 secretion, similar to that found by Hansel et al. (1991). Redmer et al. (1988) also showed increase in P_4 production in response to the same treatment, however, the effect was not significantly different from control. The stimulatory effect of this treatment has been shown to be only in small luteal cells (Hansel et al., 1991). These scientists attributed the luteotropic effects of LH and $PGF_{2\alpha}$ on small luteal cells to activation of cAMP and protein kinase C, for LH and $PGF_{2\alpha}$, respectively. Further, Hansel et al. (1991) suggested that small and large luteal cells interact with one another during the early and mid-luteal phases of the estrous cycle, whereas during the late luteal phase, the two types of cells inhibit one another. This may explain our results in which LH, $PGF_{2\alpha}$, and LH + $PGF_{2\alpha}$ had stimulatory effects only during the early and mid-luteal phases of the estrous cycle.

4.6.3. Post treatment serum P_4 production

Serum P_4 was higher during the early and mid- luteal phase than late- luteal phase in both the Ovsynch and CIDR synchronization protocols. This was in agreement with others (Armstrong and Black, 1966). Progesterone levels did not correlate with total enucleated CL weight. This was in agreement with others (Rajamahendran et al., 1976; Watson and Munro, 1980; Sprecher et al., 1989; Grazul et al., 1989), who also showed no correlation between the structure and function of the CL. The lack of correlation (found by these studies) between the size and function of the CL was found after both rectal palpation and ultrasonography of the CL and in both serum and milk samples. Functional luteolysis in the Ovsynch-treated cows started to occur on day 19, while structural luteolysis occurred on day 20 of the estrous cycle, in agreement with Erb and Stormshak

(1961) and Rao et al. (1979), who found that functional luteolysis preceded structural luteolysis. Serum P_4 concentrations showed that functional luteolysis in the CIDR cows did not occur until day 20, with structural luteolysis occurring on day 20. In agreement with our results, Rajamahendran et al. (1976) found high P_4 concentrations in two heifers, on days 18 and 20 of the estrous cycle (22-day cycle).

4.7. Conclusion

Corpora lutea formed after Ovsynch and CIDR ovulation synchronization protocols yielded similar in vitro P_4 concentrations at different stages of the synchronized cycle. The response to LH, $PGF_{2\alpha}$, and LH + $PGF_{2\alpha}$ was also not different between the two protocols. This study suggests that the observed variability in PR observed in previous studies is not due to differences in CL function.

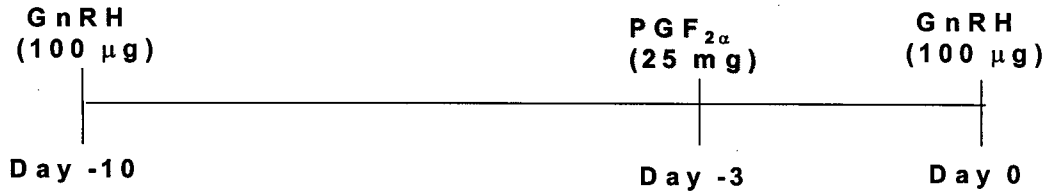

Figure 4.1a. The Ovsynch ovulation synchronization protocol which consisted of an initial injection of GnRH (100 μg), followed 7 days later by an injection of $PGF_{2\alpha}$ (25 mg) and 48 h later by a second dose of 100 μg GnRH (day = 0).

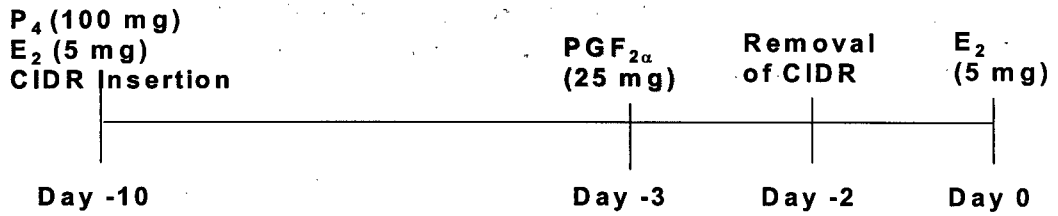

Figure 4.1b. The CIDR ovulation synchronization protocol which consisted of initial injections of P_4 (100 mg) and E_2 (5 mg) and insertion of CIDR-B® followed 7 days later by an injection of $PGF_{2\alpha}$, 24 h later removal of CIDR-B®, and 48 h after $PGF_{2\alpha}$ injection a second 5 mg dose of E_2 (day = 0).

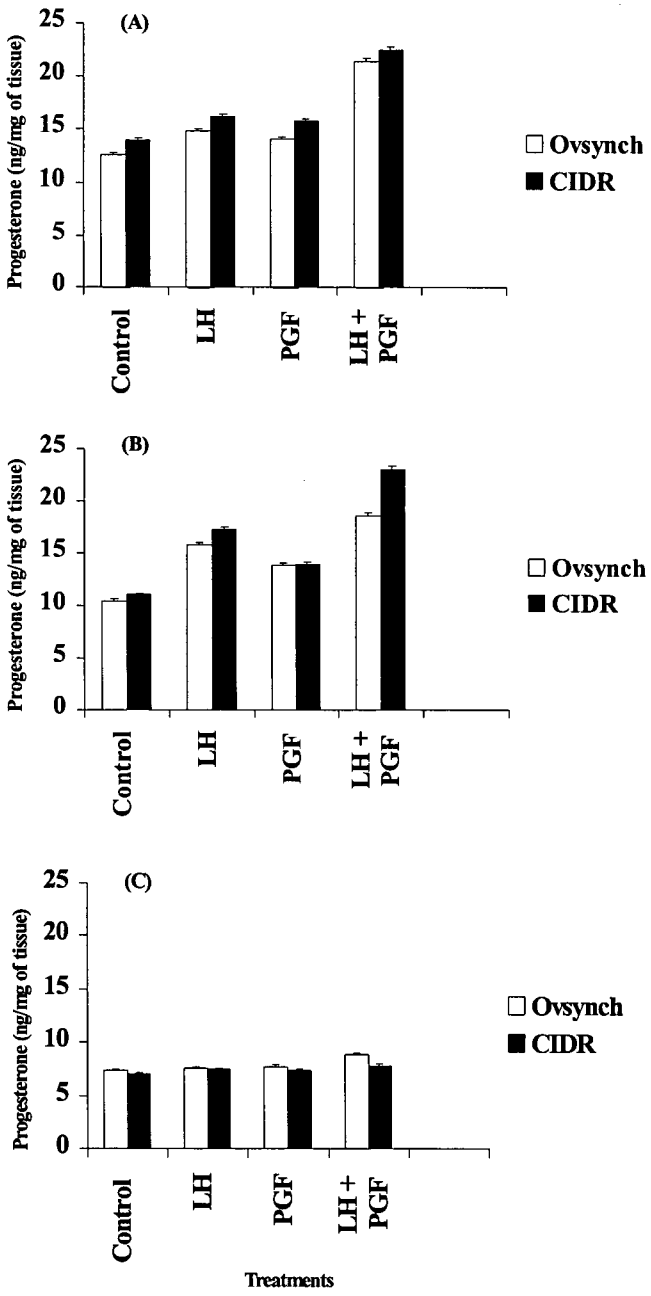

Figure 4.2. Mean progesterone (P_4) per milligram of luteal tissue ± S.E.M. after 6 h of incubation of corpora lutea (CL's) with one of four hormone treatments: none (control), LH, PGF and LH + PGF ($P > 0.05$). CL's ($n = 33$) were enucleated at day 6-8 (A), day 13-15 (B), and day 19-21 (C) of the estrous cycle following ovulation synchronization using either Ovsynch or CIDR ovulation synchronization protocols. Day 0 is 48 h after PGF, when either estradiol-17β or GnRH was given to synchronize ovulation.

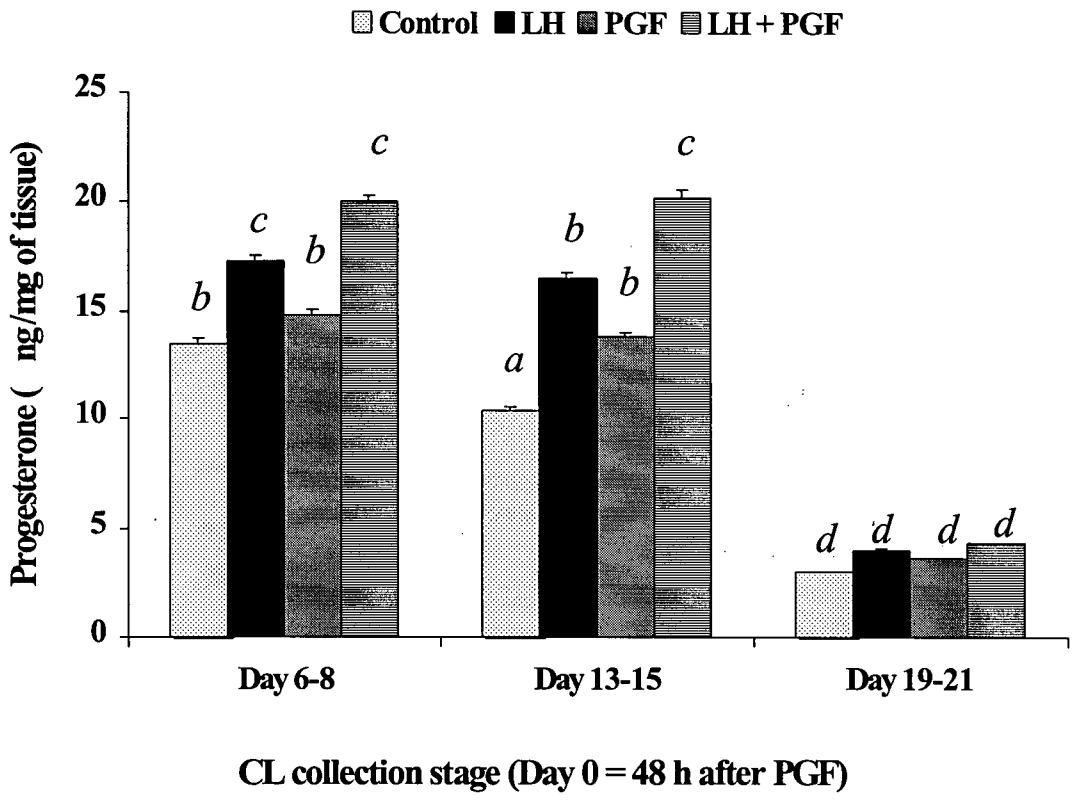

Figure 4.3. Mean progesterone (P_4) per milligram of tissue ± S.E.M. after 6 h of incubation of corpora lutea (CL) with one of four hormone treatments: none (control), LH, PGF and LH + PGF. CL (n = 33) were enucleated at day 6-8, day 13-15, and day 19-21 of the estrous cycle following the Ovsynch and CIDR ovulation synchronization protocols. Stage by treatment ($P < 0.05$). Means with different letters differ ($P < 0.05$).

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CHAPTER 5

PREGNANCY RATES AND IN VIVO PROGESTERONE PRODUCTION FOLLOWING OVSYNCH AND CIDR OVULATION SYNCHRONIZATION/ TIMED ARTIFICIAL INSEMINATION PROTOCOLS IN POSTPARTUM DAIRY COWS

5.1. Abstract

The objective of this study was to compare pregnancy rates (PR) and in vivo progesterone (P_4) concentrations following Ovsynch and CIDR ovulation synchronization/timed artificial insemination (TAI) protocols in postpartum dairy cows. Two hundred and twenty seven postpartum lactating Holstein cows were randomly divided into Ovsynch (n = 111) and the CIDR (n = 116) ovulation synchronization/TAI protocols. The Ovsynch treatment protocol consisted of an initial injection of gonadotropin-releasing hormone (GnRH; Factrel®, 100 μg), followed 7 days later by an injection of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; Lutalyse®, 25 mg) and 48 h later a second injection of Factrel® (100 μg). The CIDR treatment protocol consisted of initial injections of progesterone (P_4, 100 mg) and estradiol-17β (E_2, 5 mg) and insertion of controlled internal drug release (CIDR-B®, 1.9 g progesterone) followed 7 days later by Lutalyse® (25 mg), 24 h later removal of CIDR, and 48 h after Lutalyse® a second 5 mg injection of E_2. Artificial insemination for Ovsynch and CIDR groups of cows was performed at 64 and 76 h after $PGF_{2\alpha}$ injection, respectively. Pregnancy was diagnosed by ultrasonography at day 35 after AI and later confirmed by rectal palpation at day 60 after

AI. Milk samples were taken from the beginning of treatment (day -10) until day 35 after AI for P_4 determination. Pregnancy rates (based on ultrasound at day 35 and rectal palpation at day 60) for Ovsynch and CIDR groups of cows were 31 ± 14.9% and 42 ± 14.2%, respectively ($P > 0.05$). Ovulation synchronization rates (responder rates; based on milk P_4 levels < 1ng/ml on the day of AI and > 1ng/ml on day 7 post AI) for Ovsynch and CIDR groups of cows were 61.3 ± 13.1% and 74.8 ± 8.66%, respectively ($P > 0.05$). Therefore, based on ovulation synchronization rates, PR for Ovsynch and CIDR groups of cows were 49.4 ±14.1% and 61.9 ± 8.1%, respectively ($P > 0.05$). Pregnancy rate in primiparous cows was similar ($P > 0.05$) between the Ovsynch and CIDR treatment protocols. In multiparous cows, PR was higher ($P < 0.05$) in the CIDR protocol than the Ovsynch treatment protocols. Progesterone profiles from the time of AI until day 35 after AI were similar ($P > 0.05$) between Ovsynch and CIDR groups of cows. The apparent embryonic mortality from day 21 and 35 post AI was higher ($P < 0.05$) in the Ovsynch (32.5 ± 17.2%) than CIDR (7 ± 6.67%) cows. Ovsynch and CIDR ovulation synchronization protocols are very promising methods to eliminate estrus detection and perform fixed TAI in postpartum dairy cows to obtain optimum PR.

5.2. Introduction

There has been a steady decrease in the rate of pregnancy following artificial insemination (AI) in dairy cows; from 66% in 1950's (Spalding et al., 1974) to about 40% (Pursley et al., 1997a) in the 1990's. However, the pregnancy rate (PR) following AI in heifers has remained constant at 70% over the same time duration (Pursley et al., 1997a). A negative correlation has been reported between milk production and PR (Lean et al.,

1989; Nebel and McGilliard, 1993). Reproductive efficiency can be compromised if dairy producers aim for higher milk production, and it has a marked influence on profitability. For optimum milk production and profitability, a calving interval of 12 to 13 months has been recommended for dairy cows (Lucy and Stevenson, 1986). An integral component in achieving this calving interval is the incorporation of efficient and accurate estrus detection, proper semen handling techniques, and timely AI (TAI) relative to ovulation.

Estrus detection has been cited as one of the most important factors affecting the reproductive success of AI programs (Everett and Bean, 1986). Breeding other than at the time of estrus (when milk progesterone (P_4) concentration is greater than 1 ng/ml) may occur in 10 to 12% of all breedings and may be as high as 20 to 30% under certain management conditions (Senger et al., 1988; Rajamahendran et al., 1993). Under these circumstances, not only are the costs of maintaining the cow and purchasing and holding semen wasted, but other reproductive problems, including early embryonic mortality may result from wrongly timed inseminations (Rajamahendran et al., 2001). Thus, maximizing estrus detection can improve overall reproductive efficiency in dairy cattle. However, proper control of the time of estrus is difficult, since peak estrus activity often occurs at night and determination of the actual onset of standing estrus may be difficult without 24 h observation (Henricks et al., 1971). Estrus detection rate has been estimated to be 50% in dairy cows (Rajamahendran et al., 1993; De Rensis and Peters, 1999). To improve reproductive management and thus profitability in cattle industry, various estrus synchronization protocols have been adopted to maximize the use of AI by reducing the

time and labor involved in estrus detection, and by bringing a large percentage of a group of females into estrus at pre-determined time.

Earlier protocols have involved controlling the estrous cycle length by extending the life span of the corpus luteum (CL) by the use of progestagens (Stock and Fortune, 1993; Anderson and Day, 1998) or shortening the life span of the CL by the use of $PGF_{2\alpha}$ (Lucy et al., 1986; Stephens and Rajamahendran, 1998). Because maturity of the dominant follicle (DF) and thus ovulation varies with individual cows, estrus cannot be accurately synchronized with these synchronizing protocols. Animals show estrus 2 to 6 days following treatment with progestational compounds and fertility rates were far less than those following natural estrus (Rajamahendran et al., 2001). Further, A 7-day period of estrus detection is necessary following administration of $PGF_{2\alpha}$ 11 to 14 days apart (Lucy et al., 1986; Stephens and Rajamahendran, 1998). As a result, TAI 72 to 80 h after a second $PGF_{2\alpha}$ injection resulted in a significantly lower PR than animals inseminated after natural estrus (Stevenson et al., 1987; Stevenson et al., 1989). An increase in the basic understanding as well as the development of treatment regimes to manipulate ovarian follicular and CL dynamics over the last decade have resulted in development of estrus/ovulation synchronization protocols, namely Ovsynch (Pursley et al., 1995; Hirad et al., 1999) and CIDR + estradiol (Martinez et al., 2000; Garcia and Salaheddine, 2001), which are based on: a) elimination of the DF, b) initiation of a new follicular wave, and c) synchronization of ovulation and TAI.

The Ovsynch protocol is composed of a first gonadotropin-releasing hormone

(GnRH) injection for ovulation of the DF, a prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) injection to cause luteal regression of existing or induced CL, and a second GnRH injection to cause ovulation of the DF, following regression of CL. Pregnancy rates following Ovsynch protocol have not exceeded 40 % in dairy cows and heifers (Pursley et al., 1995; Burke et al., 1996; Pursley et al., 1997a, b; Peters and Pursley, 2003). The CIDR protocol consists of an insertion of intravaginal progesterone device (CIDR-B®) and injections of P_4 and estradiol-17β (E_2) to cause regression of the DF, followed by a $PGF_{2\alpha}$ injection to cause luteal regression of existing or induced CL, and a second E_2 injection following the removal of CIDR-B® to synchronize ovulation (Martinez et al., 2000). Pregnancy rates ranging between 30% and 80% have been reported in dairy cows and heifers after treatment with CIDR + estradiol-based ovulation synchronization protocols (Lammoglia et al., 1998; Day et al., 2000; Martinez et al., 2000; Ambrose et al., 2001; Colazo et al., 2003). (See chapter 2 for details on Ovsynch and CIDR ovulation synchronization protocols).

Progesterone is important for the transport and survival of the embryo in cattle (Garcia-Winder et al., 1986; Taylor and Rajamahendran, 1994). Cows with low P_4 concentrations between days 10 and 16 post-breeding did not maintain pregnancy (Henricks et al., 1971; Lamming et al., 1989). More data on PR is needed and information on in vivo CL function is scarce, following treatment with either Ovsynch or CIDR ovulation synchronization/TAI protocols in lactating dairy cows. Further, a direct comparison of these two treatment protocols has not been carried out in the field. Therefore, the objective of the present study was to conduct a large scale study and

compare in vivo P_4 production as well as PR between cows synchronized using the Ovsynch and CIDR ovulation synchronization/TAI protocols.

5.3. Materials and Methods

5.3.1. Animals and treatments

This study was conducted at the University of British Columbia Dairy Education and Research Centre, in Agassiz, British Columbia, Canada, between September 2000 to June 2002. Lactating Holstein dairy cows between 1 to 6 lactations and averaging 93 ± 26 days postpartum and 40 ± 9 kg milk production, were housed in free stall system that allowed a considerable amount of interaction between cows. Diets containing grass silage, corn silage, grass hay and concentrate were fed to cows throughout the study. The diet consisted of 46% dry matter (DM), 19% crude protein (CP), 7% undegradable intake protein (UIP), 11% degradable intake protein (DIP), 73% total digestible nutrient (TDN), 4% fat, 18% acid detergent fiber (ADF), 31% neutral detergent fiber (NDF), 0.92% calcium, 0.51% phosphorus, and 21% NDF forage. All handling and management of animals were in accordance with the guidelines of the Canadian Council on Animal Care (1993). Cows with injuries or history of reproductive problems were excluded from the experiment. A total of two hundred twenty-seven cows were randomly allocated into the Ovsynch (n = 111) and the CIDR (n = 116) ovulation synchronization/TAI protocols.

5.3.2. The Ovsynch protocol

Primiparous (n = 44) and multiparous cows (n = 67) were assigned to the Ovsynch treatment protocol (Figure 5.1a), which consisted of an initial GnRH injection (100 μg of Factrel®; Fort Dodge Laboratories, Fort Dodge, IA) on day -10, followed by a $PGF_{2\alpha}$ injection (25 mg of Lutalyse®; Pharmacia Animal Health, Orangeville, ON) 7 days later (day -3), and a second injection of GnRH (100 μg) 48 h after $PGF_{2\alpha}$ injection (day -1). Treatment was initiated regardless of the stage of the estrous cycle of the animals. The time of AI (day 0) was at 16 h after the second GnRH injection (64 h after the $PGF_{2\alpha}$ injection).

5.3.3. The CIDR protocol

Primiparous (n = 45) and multiparous cows (n = 71) were assigned to the CIDR treatment protocol (Figure 5.1b), which consisted of initial injections of P_4 (100 mg; Sigma-Aldrich Canada Ltd) and E_2 (5 mg; Sigma-Aldrich Canada Ltd) and insertion of controlled internal drug release device (CIDR-B®; 1.9 g progesterone, Inter-Ag, Hamilton, NZ) on day -10, followed 7 days later by 25 mg of $PGF_{2\alpha}$ injection (day -3), CIDR removal on day -2 and a second injection of E_2 on day -1. Like the Ovsynch, the CIDR treatment protocol was initiated regardless of the stage of the estrous cycle of the animals. The time of AI (day 0) was at 28 h after the second injection of E_2 (76 h after the $PGF_{2\alpha}$ injection).

5.3.4. Control cows

Data of 56 lactating Holstein cows (control; 79 ± 21 days postpartum and milk production of 41 ± 11 kgs) and representative of the same months of synchronization as in Ovsynch and CIDR synchronization protocols was later added to the statistical analyses for comparison. These cows were bred at 12 h after standing estrus was observed.

5.3.5. Milk sample collection for P_4 analysis

Milk samples were exclusive of the Ovsynch and CIDR treatment protocols, and they were collected on days -10, -3, 0, and 7, 14, 21, 28, 35, post AI, for P_4 analysis. Few milk samples occasionally were missed from the different days of milk sampling. Measurement of P_4 was conducted using a commercially available solid-phase RIA kit (Coat-A-Count; Diagnostic Products Corp., Los Angeles, CA). The kit had previously undergone validation process in our laboratory for the measurement of P_4 in cow's milk and plasma (Rajamahendran and Taylor, 1990). On the day of assay an aliquot of 100 µl of the standards was transferred into duplicate appropriate antibody coated tubes labeled A, B, C, D, E, F, and G. The standard tubes (A through G) corresponded to P_4 concentrations of 0, 0.1, 0.5, 2.0, 10.0, 20.0, and 40.0 ng/ml. Then, P_4-buffered I^{125} - labeled P_4 (1.0 ml) was added to all tubes. The tubes were then mixed and incubated at room temperature for 3 h for equilibrium. After incubation, the tubes were decanted and counted for 1 min using the gamma counter (Packard Auto gamma 500, Packard Instruments, Downers Grove, IL, USA). The intra-assay and inter-assay coefficient variations for P_4 were 9.3% and 8.7%, respectively.

5.3.6. Diagnosis of pregnancy

As described by Rajamahendran and Taylor (1990), a real-time ultrasound scanning instrument (Aloka 500 V, Aloka Co. Ltd., Tokyo, Japan) equipped with 7.5 MHz transrectal transducer was used on day 35 (day 45 from the start of treatment) after AI, to examine the uteri of the cows for the presence of embryos. All cows were later palpated at 60 days post AI for confirmation of pregnancy.

5.4. Statistical analysis

For the Ovsynch and CIDR- treated cows, PR was defined as the percentage of cows that became pregnant in relation to the total number treated. Animals that were pregnant at day 35 post AI were also pregnant at day 60 when they were rectally palpated. Therefore, day 35 pregnancy data was used in the statistical analysis to compare PR. Ovulation synchronization rate (responder rate) was determined by cows having P_4 levels < 1 ng/ml on day of AI and P_4 > 1 ng/ml on day 7 post AI. Contingency tables in the chi-square analysis were used to compare: 1) the overall PR between Ovsynch and CIDR, 2) the proportion of animals which responded to treatments (ovulation synchronization rate), 3) the proportion of responder cows that became pregnant within treatment (Ovsynch and CIDR), 4) overall PR between primiparous and multiparous cows, 5) PR based on month (animals were not synchronized in June and November) of year, 6) proportions of cows with P_4 < 1 ng/ ml on day 0, > 1 ng/ml on days 7, 14, and 21 post AI, and 7) estimated embryonic mortality based on animals with P_4 levels < 1 ng/ml on day 0, and > 1 ng/ml at days 7, 14 and 21 and ultrasound on day 35 post AI. Least-square analysis of variance using the JMP IN® statistical package (2001) was used to

analyze CL function (based on P_4 levels on days 0, 7, 14, 21, 28, and 35 post AI) in the responder cows (both pregnant and non-pregnant) of the Ovsynch and CIDR treatment protocols. Student-t test was used to compare differences between means with significant difference. The level of significance was set at $\alpha = 0.05$.

5.5. Results

5.5.1. Pregnancy rates

The combined PR between primiparous and multiparous cows based on ultrasound and rectal palpation between the Ovsynch (31 ± 14.9%), CIDR (42 ± 14.2%) cows were similar ($P > 0.05$; Figure 5.2a). Ovulation synchronization rates for Ovsynch and CIDR groups of cows were 61.3 ± 13.1% and 74.8 ± 8.66%, respectively ($P > 0.05$; Figure 5.2b). Therefore, based on ovulation synchronization rates, PR for Ovsynch and CIDR groups of cows were 49.4 ± 14.1% and 61.9 ± 8.1%, respectively ($P > 0.05$; Figure 5.2c). Pregnancy rate in primiparous cows was similar ($P > 0.05$) between the Ovsynch and CIDR treatment protocols (Table 5.1). In multiparous cows, PR was higher ($P < 0.05$) in the CIDR protocol than the Ovsynch treatment protocol (Table 5.1). Aside from the month of February, in which PR was higher ($P < 0.05$) in the CIDR than Ovsynch, PR were similar ($P > 0.05$) between the Ovsynch and CIDR treatment protocols throughout the months of synchronization. The CIDR treatment protocol resulted in low PR for January and March (17% PR), otherwise, the CIDR protocol resulted in 56% PR in February and from 35% to 61% between April to October (Figure 5.3a). Pregnancy rates following the Ovsynch protocol showed more fluctuation during the months of synchronization, with PR being lower from January to April, July and October (Less than

31%), whereas during the months of May, August, September, and December, respectively, PR averaged 33%, 47%, 50%, and 57% (Figure 5.3a). In the control cows, PR was similar ($P > 0.05$) from December to April and from May to October (Figure. 5.3b).

5.5.2. Corpus luteum function

In the responder cows, P_4 profiles from the time of AI until day 35 following AI were similar ($P > 0.05$) between Ovsynch and CIDR groups of cows ($P > 0.05$ Figure 5.4). The pregnant cows in the two protocols had higher ($P < 0.05$) P_4 levels on days 21, 28 and 35 after AI than non-pregnant cows. The presumptive PR (based on $P_4 < 1$ ng/ml on day 0, and > 1 ng/ml on days 7, 14 and 21 after TAI) was similar ($P > 0.05$) in the Ovsynch and CIDR groups of cows (Table 5.1). However, the estimated embryonic losses between days 21 to 35 after AI were higher ($P < 0.05$) in the Ovsynch than the CIDR cows (Table 5.1).

5.6. Discussion

This study compared PR and in vivo P_4 production in lactating dairy cows following treatment with the Ovsynch and CIDR ovulation synchronization and TAI protocols. Both synchronization protocols were administered at random stages of the estrous cycle and aimed to: 1) synchronize the emergence of a new follicular wave and ovulation, and 2) maximize the use of AI by saving time and labor, two components that are extensively exhausted in breeding operations dependent upon copious estrus detection.

5.6.1. Pregnancy rates

The PR of the primiparous and multiparous cows combined was similar between the CIDR and Ovsynch treatment protocols. The PR following the CIDR protocol (42%) was similar to that observed in beef cows treated with estradiol benzoate (EB) (43%, Colazo et al., 2003), higher than that involving beef cows and treated with EB (30%, Lammoglia et al., 1998; Cutaia et al., 2003; Figueiredo et al., 2003) and lower than that of beef as well as dairy cows treated with E_2 or EB (60%-80%, Martinez et al., 2000; Day et al., 2000). Pregnancy rates following the Ovsynch protocol were similar to other reports in lactating dairy cows (Pursley et al., 1995; Burke et al., 1996; Pursley et al., 1997a, b; Peters and Pursley, 2003). The reported PR in these reports were from 27 to 39%. The stage at which the treatment protocol is initiated, the time of AI following the second injection of the treatment protocol, and concentrations of E_2, are some of the factors that may have contributed to the PR obtained in this experiment following the Ovsynch and CIDR ovulation synchronization/TAI protocols.

The stage of the estrous cycle at which ovulation synchronization treatment is initiated could determine the response to treatment and therefore PR. Treatment with P_4 and E_2 together consistently have caused regression of the large follicle, stimulated emergence of new follicular wave 3-5 days after treatments (Bo et al., 1994; Rajamahendran and Manikkam, 1994). Failure of cows to respond to these treatments could result in development of persistent follicles (Savio et al., 1993). The persistence of a large/DF is accompanied with high levels of E_2, lower P_4 concentrations, and an increase in pulsatile release of LH (Sanchez et al., 1995; Kojima et al., 1995). Fertility is

reduced as a result of ovulation of persistent follicles and ovulation of aged oocytes (Ahmad et al., 1995). Initiating CIDR + P_4 + E_2 treatment at late diestrus may not lead to regression of the DF, however, this could result in reduced PR following TAI as a result of ovulation of aged oocytes (Martinez et al., 2000; Colazo et al., 2003). However, the ovulation synchronization rate to the second E_2 injection in this study was at 75% and PR in the responder cows was 61%. This was comparable to studies in dairy cows (Ryan et al., 1995), dairy heifers (Ambrose et al., 2001), beef heifers and beef cows (Martinez et al., 2000), which used either E_2, EB or estradiol cypionate (ECP).

The effectiveness of the Ovsynch protocol is also influenced by the stage of follicular development at the time of treatment (Vasconcelos et al, 1999). In this study, the ovulation synchronization rate to the second GnRH injection was 61%, lower than those reported by others (Vasconcelos et al., 1999; Mialot et al., 2003; Peters and Pursley, 2003), who reported ovulation synchronization rates between 77 and 89%. Vasconcelos et al. (1999) reported ovulation rate of 23% after the first GnRH injection, however, 96% response rate to the first GnRH injection was also reported by the same authors when treatment was given between days 5 to 9 of the estrous cycle. As a result, the ovulation synchronization rate based on the response to the second GnRH injection varied; 92% when the animals ovulated versus 79% when animals did not ovulate after the first GnRH injection (Vasconcelos et al., 1999). In this study, 49% became pregnant out of the cows, which responded to the second GnRH injection (61%), Thus, this rate of pregnancy could be a result of 39% of the cows, which did not respond to the first GnRH injection. Further, Vasconcelos et al. (1999) reported that 6% of cows ovulated before the

second injection of GnRH and these cows were in the late diestrus stage of the estrous cycle, the time when they would be expected to have normal luteal regression prior to $PGF_{2\alpha}$ treatment. The same authors also found that 68% of the cows, which had low serum P_4 at the time of $PGF_{2\alpha}$ injection had a synchronized ovulation (ovulated after the second GnRH injection), similar to the synchronized ovulation rate (61%) obtained in this study. It has been reported that lactating dairy cows synchronized with Ovsynch treatment protocol developed larger diameter of DF's, which were less estrogenic, and collectively that might have contributed to lower PR (de la Sota et al., 1993; Vasconcelos et al., 1999).

The fertile life of sperm is between 24 to 48 h and the fertile life of the ovum is between 6 to 24 h (Sorensen, 1979). In this study, the cows in the Ovsynch protocol were artificially bred at 16 h after the second GnRH injection. Ovulation in lactating cows occurs between 24 and 32 of the second GnRH injection (Pursley et al., 1995). Therefore, if the sperm life span is only 24 h and ovulation occurs at 48 h, fertilization would not occur; however, if the life span of the sperm is 48 h and ovulation occurs at 48 h after the second GnRH injection, fertilization may occur. The LH peak and behavioral estrus occur between 14 and 32 h after treatment with EB (Beck and Convey, 1977; Zaied et al., 1981; Hanlon et al., 1996). Therefore, the time of AI in the CIDR protocol (28 h after second E_2) was appropriate, based on the expected time of ovulation between 42 to 56 h following E_2 injection.

Bo et al. (2000) indicated that elevation of E_2 for a period between 12 h (0.5 or 1 mg) to 42 h (5 mg) is necessary to cause atresia of the DF. In dairy cows, the use of 1 mg of EB was not as effective in inducing follicle suppression when compared to 2 mg of EB (Day et al., 2000). The administration of GnRH results in the secretion of LH, which in turn results in increase in E_2 concentrations in circulation (Stevenson et al., 1993). The concentration of E_2 in the follicular fluid has been measured to be approximately 15 microgram (Singh et al., 1998). Estradiol from the preovulatory follicle initiates luteolysis by releasing of neurohypophysial oxytocin which triggers the release of uterine $PGF_{2\alpha}$ and thus luteal regression (Niswender et al., 2000). Estradiol initiates the process of luteolysis around days 12 to 14 of the estrous cycle (Mann and Lamming, 2000). Estradiol has been found to be higher in pregnant heifers compared to non-pregnant heifers between days 6 and 16, post estrus (Lukaszweska and Hansel, 1980). Moreover, the receptors of E_2 have been found to be highest at the time of estrus (Wathes and Hamon, 1993). The 5 mg dose of E_2 used in this study is about 333 times more than that present in the follicle, and this dose causes elevation of E_2 in circulation for up to 42 h (Bo et al., 2000). It is possible that the resulting E_2 from the second GnRH injection (Ovsynch) or from the second E_2 injection of the CIDR treatment protocol may up-regulate the expression of its own receptors (Ing and Tornesi, 1997), increase endometrial oxytocin receptors (Hixon and Flint, 1987), advance the process of luteal regression, resulting in lower PR. Administration of 2 mg of E_2 following the removal of the progestagen implant decreased PR in beef heifers (Wiltbank et al., 1971). Abnormal embryonic development and reduced fertility has been attributed to prolonged exposure to E_2, both in cows (Breuel et al., 1993; Wehrman et al., 1993), and rats (Butcher and

Pope, 1979). Johnson and Lewis (1993) related lower conception rate (CR) in sheep to elevated estradiol concentration. Further, abortion in women was partly related to high levels of estradiol in follicular fluid and in serum per follicle (Lewinthal et al., 1987). However, Fike et al. (1997) found normal fertility of oocyte released from a persistent follicle.

5.6.2. Corpus luteum function

Progesterone concentrations from milk sampled on days 0, 7, 14, 21, and 35 were not different between the Ovsynch and CIDR treatment protocols. Irregular estrous cycles lengths, imprecise detection of estrus and ovulation and early embryonic mortality, all could lead to higher P_4 concentration at 21 days post AI (Chebel et al., 2003). Progesterone levels in the pregnant cows, however, were significantly higher on days 21, 28, and 35 post AI than in the non-pregnant cows, in both the Ovsynch and the CIDR group of cows. This agrees with those of Lamming et al. (1989) in that P_4 levels in the pregnant cows showed a consistent rise in from day 0 to day 35 post AI. The results, however, do not agree with these authors and Lucy and Stevenson (1986) in that P_4 profile in the pregnant cows in this study was not different from that of non-pregnant on days 0, 7 and 14 post AI; however, P_4 in the CIDR cows tended to be higher on days 7 and 14 post AI than Ovsynch cows. The apparent embryonic mortality between days 21 to 35 post AI after the CIDR treatment protocol (7%) was significantly lower than that found after Ovsynch treatment protocol (32%). This rate of embryonic mortality after Ovsynch was comparable to that by Vasconcelos (1999) who reported 24% embryonic loss between 25 days post AI and calving time. Loss of CL maintenance around time of

implantation could prevent implantation and could lead to loss of pregnancy. Further, Thatcher et al. (2001) reported that 40% of the total embryonic mortality occurring between days 8 and 17 of pregnancy, and this may be attributed to the inability of certain conceptuses to secrete interferon-tau (IFN-t) and inhibiting the uterine $PGF_{2\alpha}$ during early pregnancy (Thatcher et al., 2001). In this study, the animals that were diagnosed pregnant on day 35 after AI were confirmed pregnant at 60 days of gestation.

5.6.3. Effect of month on synchronization

Temperature in the Vancouver area during the summer months ranges from 16 to 25 °C and from -2 to 9 °C during the winter (Taylor and Rajamahendran, 1991). From the temperature-humidity index table presented by Armstrong (1994), the highest temperature (25 °C) in Vancouver should be in the no stress zone and should not cause any detrimental effect on fertility. Accordingly, the results of this study demonstrated that the reproductive performance based on PR over the months of synchronization were similarly variable and consistent between non synchronized (control) and cows synchronized with either the Ovsynch or the CIDR ovulation synchronization/TAI protocols. This was in agreement with Tenhagen et al. (2001) who found similar results with control cows and cows treated with the Ovsynch treatment protocol. Our results, however, are in contrast to reports by others in Florida (Burke et al., 1996; de la Sota et al., 1998). These authors found less variability in PR with the use of Ovsynch in comparison to control from January through April, or when Ovsynch was compared to control in the summer months (May to September). The weather in Florida, especially in the summer months can be much warmer and more humid than that of Vancouver.

Between December and April, all cows are kept indoors, as opposed to the time period between May and October, when cows are sometimes kept out on the pasture. Although not significant, more pregnancies were observed in the control cows between December and April, relative to the months between May and October. Whether or not an increase in the number of cows sampled would make the results significant is unclear. However, cows can be monitored and heat checked more frequently when they are kept indoors, especially in dairy operations.

5.6.4. Effect of days postpartum on synchronization and pregnancy

Both the Ovsynch and CIDR treated cows were cycling (82% vs. 100%), which did not appear to affect pregnancy. Uterine involution (decrease in size of uterine horns, sloughing of old tissue, and renewal of uterine epithelium (Kiracofe, 1980) is usually completed between 30 to 60 days postpartum (Wagner and Hansel, 1969; Gier and Marion, 1968). Thus, days postpartum (> 70 days for all groups) should not have influenced PR in this experiment. Pursley et al. (1997a) has found that lactating multiparous cows of > 76 days postpartum had higher PR than cows between 60 to 75 days postpartum, following Ovsynch treatment protocol. This does not agree with our results, in which we found that multiparous cows between 60 to 90 days postpartum had similar PR to those of > 90 days postpartum, and therefore, the suggestion (Nebel and Jobst, 1998) that extension of the voluntary waiting period to at least 75 days for the Ovsynch protocol would optimize PR was not supported by the results of this study. The same conclusion can be said about the CIDR treatment protocol, in that days postpartum had no effect on PR in this experiment. The CIDR treatment protocol resulted in similar

PR to the Ovsynch treatment protocol, in cows between 60 to 90 (42% vs. 29%) and > 90 (40% vs. 26%) days postpartum.

5.6.5. Pregnancy rates between primiparous and multiparous cows

There was no significant difference in PR between primiparous and multiparous lactating cows, in contrast to Tenhagen et al. (2001), who found higher CR in primiparous than multiparous cows following treatment with Ovsynch ovulation synchronization/TAI protocol. Our finding is also in contrast to results by others in which it was found that first parity cows had higher CR than older cows (Lean et al., 1989; Folman et al., 1990; Eicker et al., 1996). Older cows (multiparous) consume more DMI during postpartum period than younger cows (heifers and primiparous) (Lucy et al., 1992; Grant and Albright, 1995). This is attributed to larger sizes and social dominance which multiparous cows usually possess over primiparous cows, especially when cows are housed in a free stall type of facility (Grant and Albright, 1995). The cows in this study were housed in a free stall type of facility and were able to interact freely. However, the type of interaction and facility did not appear to affect PR. An increase in the DMI can be a determining factor of both energy intake and balance (Chase, 1993). Lucy et al (1992) found that multiparous cows consumed more DMI and had a more positive energy balance, which may have contributed to earlier ovulation and higher PR than primiparous cows. Thus, multiparous cows may have higher social dominance hierarchy, more DMI, more milk, more positive energy balance, which may advance ovulation and enhance PR. The PR in multiparous cows was significantly higher in the CIDR protocol compared to Ovsynch (45% vs. 27%). This is encouraging to dairy producers because they can gain

more profits by using the CIDR protocol with high milk yielding cows. On the other hand, we must be cautious with this finding, because this could be as a result of higher PR in the month of February following the CIDR relative to Ovsynch treatment protocol.

5.6.6. Effect of milk production on P_4 and pregnancy

In this study, there was a significant and linear trend between milk production and parity. As parity increased, milk production increased (mostly for the first three lactations), similar to the finding by Tenhagen et al. (2001). Adverse effects of high peak milk yields on early pregnancy have been noted (Lean et al., 1989). Increase in milk production also increased follicle size diameter (Vasconcelos et al., 1999). These same authors also reported reduced serum P_4 16 days post Ovsynch and this was positively correlated with milk production. This is similar to the findings of the present experiment in which milk production significantly affected P_4 concentrations; as milk production increased, P_4 concentrations decreased. Based on this, high milk production at 21,28, and 35 days post AI may have contributed to the embryonic loss and PR obtained following synchronization with the Ovsynch and CIDR treatment protocols.

5.7. Conclusion

The data of this experiment demonstrated that in both the Ovsynch and CIDR ovulation synchronization/TAI protocols, PR were substantially higher in the animals, which had synchronized ovulations. The data also demonstrated that the CIDR ovulation synchronization/TAI protocol resulted in higher PR in multiparous cows relative to the Ovsynch ovulation synchronization/TAI protocols. Also, cows treated with the CIDR

treatment protocol had lower apparent embryonic mortality between days 21 and 35 post AI, when compared to the Ovsynch treatment protocol. However, both the Ovsynch and CIDR ovulation synchronization protocols can be used throughout the year and regardless of the stage of the animal's estrous cycle, and both procedures can be effective for synchronization of ovulation and TAI programs. More efforts need to be put into improving these methods to achieve maximum PR. For the Ovsynch protocol, the future experiments should focus on: 1) supplementation of exogenous progestins during the synchronization period to minimize or prevent premature estrus or ovulation, which often occur when treatment is initiated at late diestrus, 2) presynchronization with $PGF_{2\alpha}$ 12 to 14 days apart, so that when the Ovsynch is initiated the cows will be at diestrus stage of the estrous cycle, the time which has lead to highest PR, and 3) a combination of presynchronization with $PGF_{2\alpha}$ and exogenous progestins. With regard to the CIDR protocol, experiments should focus on: 1) reducing the dose of the second E_2 to minimize the chance of luteolysis and termination of pregnancy, and 2) initiating the CIDR protocol at times other than late diestrus, and this could be achieved by presynchronization with $PGF_{2\alpha}$ 12 to 14 days a part.

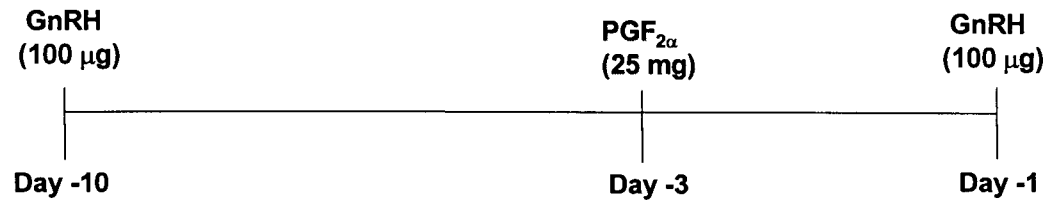

Figure 5.1a. The Ovsynch ovulation synchronization/TAI protocol, which consisted of an initial GnRH injection (100 μg) on day -10, followed by a $PGF_{2\alpha}$ injection (25 mg) 7 days later (day -3), and a second injection of GnRH (100 μg) 48 h after $PGF_{2\alpha}$ injection (day -1). The time of AI (day 0) was at 16 h after the second GnRH injection (64 h after the $PGF_{2\alpha}$ injection).

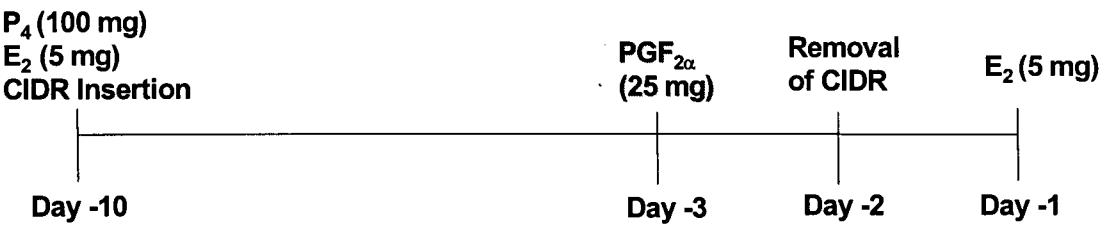

Figure 5.1b. The CIDR ovulation synchronization/TAI protocol, which consisted of initial injections of P_4 (100 mg) and E_2 (5 mg) and insertion of (CIDR-B®) on day -10, followed 7 days later by 25 mg of $PGF_{2\alpha}$ injection (day -3), CIDR-B® removal on day -2 and a second injection of E_2 on day -1. The time of AI (day 0) was at 28 h after the second injection of E_2 (76 h after the $PGF_{2\alpha}$ injection).

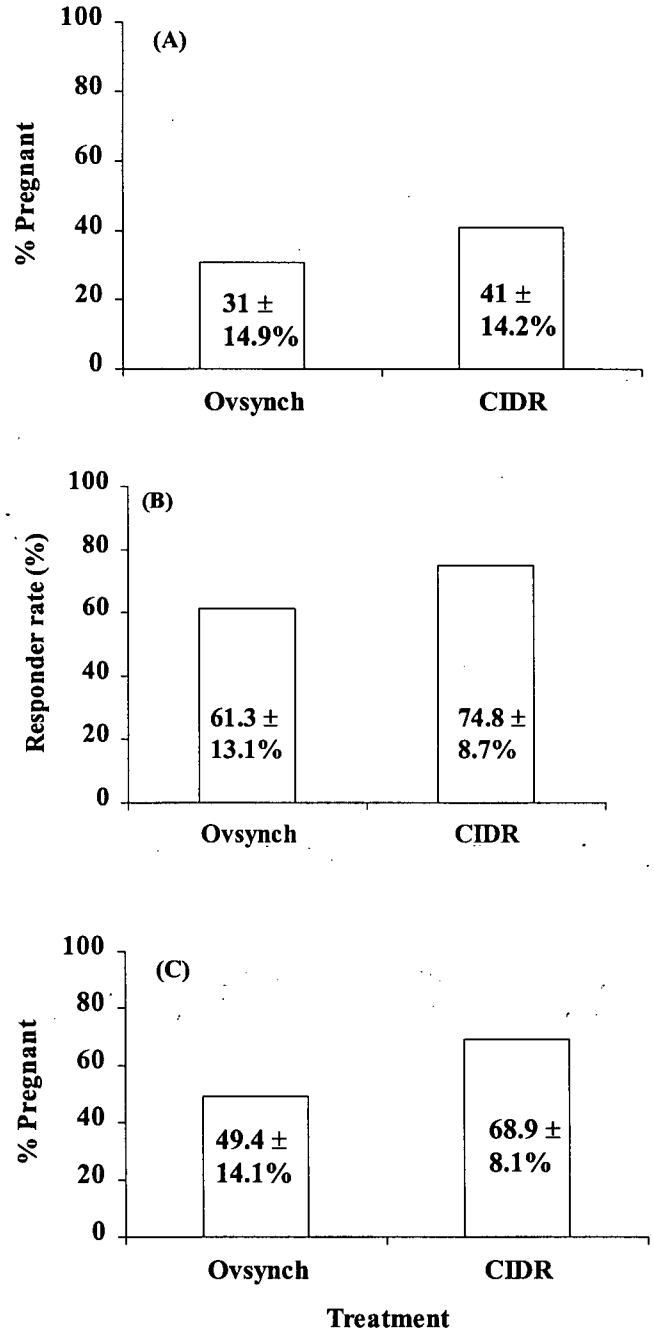

Figure 5.2. (A) Pregnancy rates in lactating dairy cows synchronized with Ovsynch and CIDR ovulation synchronization protocols ($P > 0.05$). (B) Ovulation synchronization rate (responder rate) in cows synchronized with Ovsynch and CIDR ovulation synchronization protocols ($P > 0.05$). (C) Pregnancy rate based on responder rate following Ovsynch and CIDR treatment protocols ($P > 0.05$).

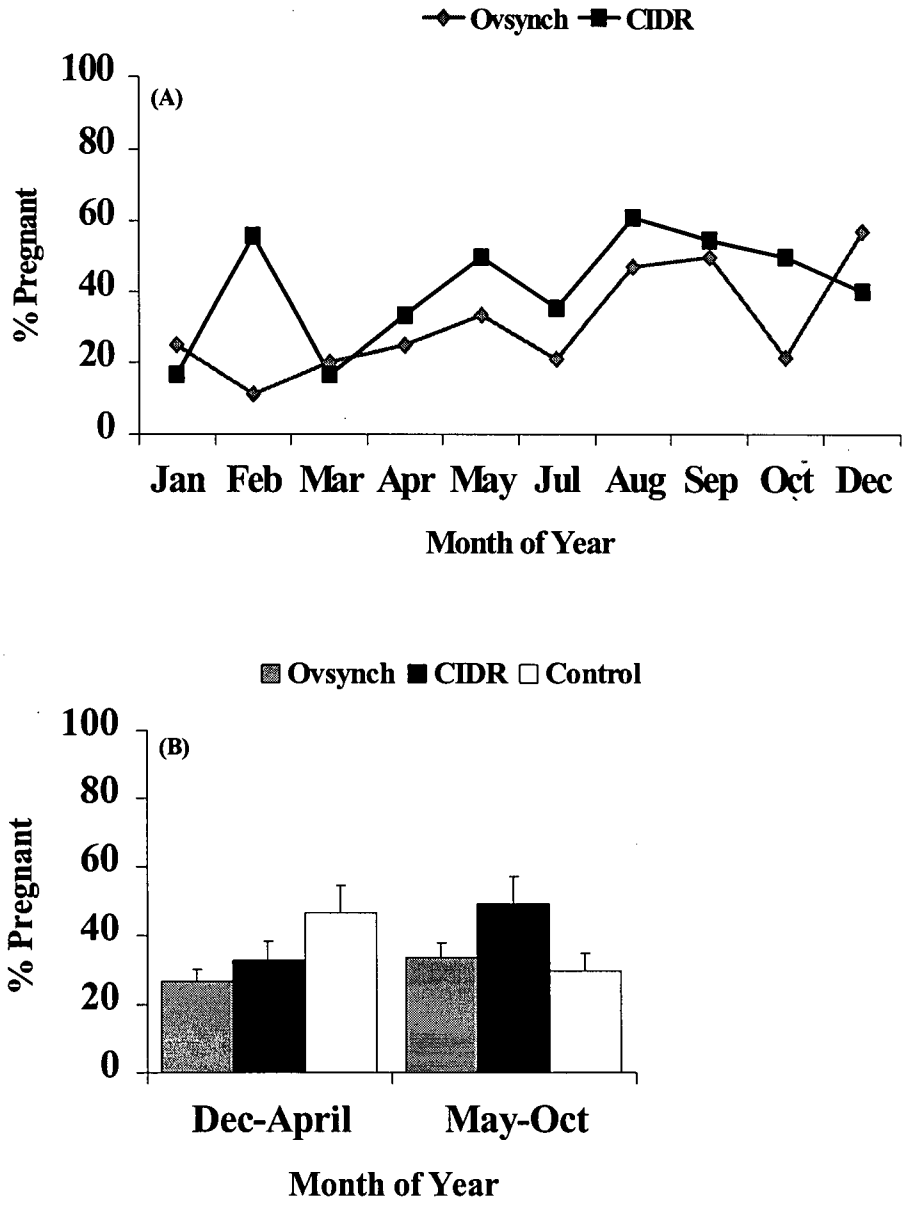

Figure 5.3. (A) Pregnancy rates over the period of ovulation synchronization (September 2000 to June 2002) in cows synchronized with Ovsynch and CIDR ovulation synchronization/TAI protocols ($P > 0.05$). (B) Pregnancy rates between Ovsynch, CIDR and control cows between December to April and May to October ($P > 0.05$).

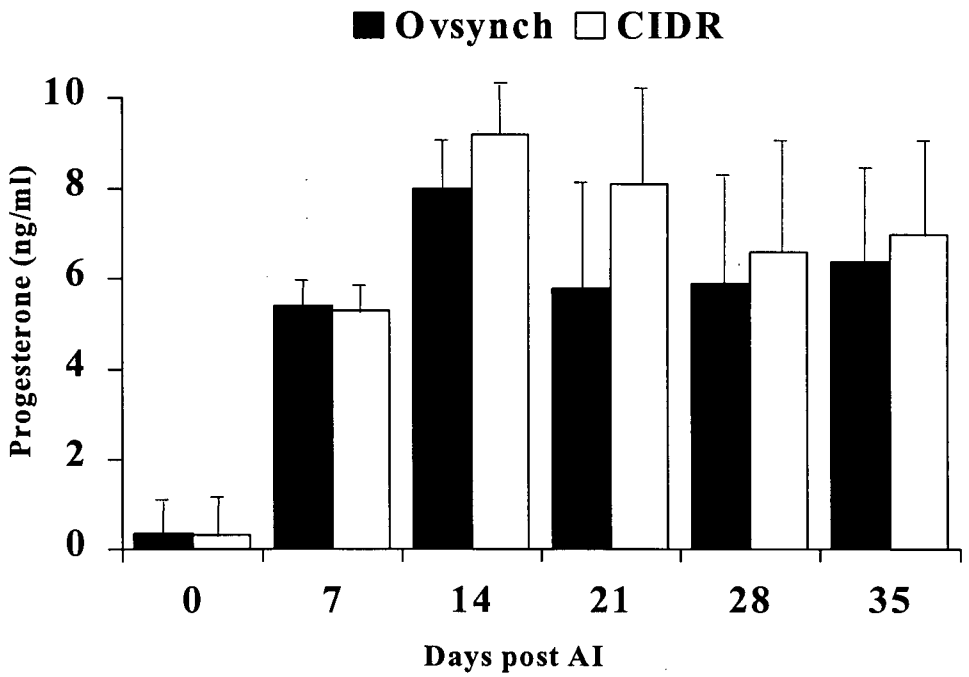

Figure 5.4. Milk progesterone (ng/ml) in responder cows (cows having $P_4 < 1.0$ ng/ml on day 0 and > 1.0 ng/ml on day 7 post AI) following the Ovsynch and CIDR ovulation synchronization/TAI protocols. Milk samples were collected on days 0, 7, 14, 21, 28, and 35 post AI ($P > 0.05$).

Table 5.1. Pregnancy rates in primiparous and multiparous non-synchronized (control) cows inseminated after natural estrus and cows inseminated upon appointment following the CIDR and Ovsynch synchronization/TAI protocols, presumptive pregnancy (based on $P_4 < 1$ ng/ml on day 0, and $P_4 > 1$ ng/ml on days 7, 14 and 21 post AI), and estimated embryonic mortality (difference between day 21 and day 35 AI pregnancy rate). Different letters represent significant difference. ($\alpha = 0.05$)

	Ovsynch	CIDR	Control
Pregnancy rate in primiparous cows (%)	34.1±1.6[a]	37.8 ± 0.8[ab]	44±1.0[a]
Pregnancy rate in multiparous cows (%)	26.9±1.6[a]	45.0 ± 2.2[bc]	27.6± 0.79[abc]
Presumptive AI Pregnancy (21 day) (%)	74.3a	64.5a	
Estimated Embryonic Loss (%)	32.5a	7.0b	

[a] = P > 0.05

[b] = P < 0.05

[bc] = P < 0.05

[abc] = P > 0.05

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CHAPTER 6

GENERAL DISCUSSION, SUMMARY AND CONCLUSIONS

6.1. General discussion

In a normally managed herd, cows/heifers would be at different stages of the estrous cycle, which means that a follicular wave would be emerging in some animals while other animals would have dominant follicles (DF's) capable of immediate maturation and ovulation. The timing of synchronized estrus and ovulation would be variable if follicular development in such a mixed population is not controlled, which collectively could adversely affect time of artificial insemination (AI) and subsequent fertility rate. Development of protocols that induce the development of a new follicle would therefore lead to the improvement of estrus/ovulation synchronization by assuring that the ovulatory follicle has the optimum potential for fertilization, adequate corpus luteum (CL) function, embryo development and pregnancy rates (PR).

Ovsynch and CIDR are emerging protocols that are aimed to initiate a new follicular wave by eliminating the DF, with the aim of synchronizing ovulation thereby facilitating AI of cattle at a fixed time (TAI). The Ovsynch treatment protocol has yielded higher PR in dairy cows than dairy heifers (Pursley et al., 1995). Pregnancy rates in heifers obtained following synchronization with Ovsynch treatment protocol (40%; Ambrose et al., 2000) is considerably lower than what could be achieved with breeding following natural estrus (≤ 70%; Pursley et al., 1997a). However, PR of 60-80% have

been reported in heifers and cows following synchronization with CIDR treatment protocols (Macmillan and Burke, 1996; Martinez et al., 2000; Martinez et al., 2002). This study researched LH responses, CL formation and function, ovarian follicular dynamics, and PR following Ovsynch and CIDR treatment protocols for ovulation synchronization. This information would enable us to modify and recommend these synchronization protocol(s) for cattle producers, based on cost and practicality. This was the first study in which a direct comparison was made between the Ovsynch and CIDR ovulation/TAI treatment protocols. The study involved both in vivo and in vitro experiments and included beef cows, and dairy heifers and cows.

In this study, similar post synchronization LH profiles, follicular dynamics, progesterone (P_4) profiles and PR following Ovsynch and CIDR synchronization protocols suggested that both protocols can be equally effective in synchronization of ovulation, elimination of estrus detection and enhancement of pregnancy in heifers as well as cows. In Chapter 3, we investigated in dairy heifers the effects of the Ovsynch and CIDR ovulation synchronization/TAI protocols on LH profiles, follicular dynamics, CL function and PR post ovulation synchronization. The results demonstrated that both protocols resulted in high ovulation synchronization rates (83.3% and 100% for the Ovsynch and CIDR, respectively), P_4 concentrations and PR regardless of the stage of the estrous cycle when treatments are initiated. Chapter 3 experiment also demonstrated that low ovulation rates in response to the first GnRH injection in the Ovsynch protocol did not influence ovulation synchronization rates following the second GnRH injection, which was in contrast to that found by others (Pursley et al., 1995; Pursley et al., 1997a,b;

Vasconcelos et al., 1999; Moreira et al., 2000; Cavestany et al., 2003; Peters and Pursley, 2003). However, initiation of Ovsynch protocol late in the estrous cycle appears to cause lower ovulation synchronization rates as well as PR as a result of premature ovulation, in agreement with other findings (Vasconcelos et al., 1999; Moreira et al., 2000; Cavestany et al., 2003). The experiment also illustrated that diameter of the ovulatory DF, and duration of dominance of the ovulatory follicle had no effects on subsequent LH profiles, follicular dynamics, P_4 concentrations, or PR between the Ovsynch and CIDR ovulation synchronization/TAI protocols. This is in contrast to other findings in which diameter and duration of the DF resulted in either lower or higher subsequent P_4 concentrations and lower or higher PR (Snachez et al., 1995; Vasconcelos et al., 1999; Moreira et al., 2000; Vasconcelos et al., 2001). Our findings, however, imply that incidences of premature ovulation and thus lower PR in heifers following Ovsynch synchronization protocol could be as a result of premature LH surge prior to the second GnRH injection, which could adversely affect time of AI and subsequent fertility rate. Further, PR following the Ovsynch protocol were higher than those reported in other studies using heifers (Pursley et al., 1995; Pursley et al., 1997b). Pregnancy rates observed after the CIDR ovulation synchronization were comparable to those reported in beef heifers (Martinez et al., 2000) or dairy heifers (Ambrose et al., 2001).

Subluteal function has been shown to be associated with reduced fertility (Bulman and Lamming, 1978). Therefore, emphasis was put in this study on the effect of both the Ovsynch and CIDR ovulation synchronization protocols on P_4 profiles following synchronization. These data (both in vivo & in vitro experiments) showed that P_4

production following synchronization did not differ between the Ovsynch and CIDR ovulation synchronization protocols and the results were consistent in beef cows, dairy heifers and dairy cows. Pregnancy rates following synchronization were also similar between the two treatment protocols. This supports the positive relationship between PR and P_4 profiles in cattle (Lamming et al., 1989). Pregnancy rates in lactating dairy cows following the Ovsynch protocol (Chapter 5) were similar to some studies (Burke et al., 1996; Peters and Pursley, 2003) and dissimilar to other studies, in which PR averaged about 40% (Pursley et al., 1997a,b; Fricke and Wiltbank, 1999; Ambrose et al., 1999; Ambrose et al., 2000). Pregnancy rate following the CIDR protocol was comparable to those reported in dairy cows by Macmillan and Burke (1996) and different from that reported by Day et al. (2000). In the present study, PR in lactating cows following either Ovsynch or CIDR protocols were similar to those detected in non-synchronized cows (control); however, it is clear from the results that these rates of pregnancy were low and more importantly, lower than is desired or targeted by dairy producers. However, there has been a steady decrease in the rate of pregnancy following TAI in dairy cows; from 66% in 1950's (Spalding et al., 1974) to about 40% (Pursley et al., 1997a) in the 1990's. A negative correlation has been reported between milk production and PR (Lean et al., 1989; Nebel and McGilliard, 1993).

Reproductive efficiency can be compromised if dairy producers aim for higher milk production. Data from Chapter 5 demonstrated that days postpartum, parity, weather and milk production had no influence on PR, both in cows synchronized with Ovsynch or CIDR treatment protocol. Vasconcelos et al. (1999) reported that serum P_4 16 days post

Ovsynch positively regressed on increasing milk production. This is similar to the findings of Chapter 5 in which milk production significantly affected P_4 concentrations; as milk production increased, P_4 concentrations decreased. Based on this, the high levels of milk production observed at 21, 28, and 35 days post AI may have contributed to the embryonic loss and PR obtained following synchronization with the Ovsynch and CIDR treatment protocols. Further, Fricke and Wiltbank (1999) reported that higher incidence of double ovulation with high milking cows, and this according to the researchers may have contributed to the embryo losses. Nutrition and negative energy balance have been associated with reduced PR as well (Sklan et al., 1994; Roche et al., 2000). Whatever the reason for similar PR between control and synchronized cows, the present study demonstrated that either the Ovsynch and CIDR ovulation synchronization/TAI protocols could be used in cattle operations as a replacement for synchronization protocols that are based on estrus detection.

The present study, however, emphasizes the need to modify the Ovsynch and CIDR ovulation synchronization protocols in order to achieve optimum ovulation synchronization rate, CL function and PR. These modifications should be based on practicality and cost. As noted earlier the stage of the estrous cycle at which the Ovsynch protocol is initiated determines the success of the protocol. A drawback of the Ovsynch protocol is premature estrus and ovulation prior to the second injection of GnRH, and this occurs primarily when Ovsynch is initiated at the late diestrus stage of the estrous cycle. To overcome this problem, exogenous P_4 or progesterone-based devices have been included in the Ovsynch protocol. Ambrose et al. (2001) achieved 61% PR following

timed AI after including CIDR-B® in the protocol. However, Lopes et al. (2000) achieved 39% PR following TAI after using melengestrol acetate (MGA). Cavestany et al. (2003) stated that the addition of medroxyacetate progesterone (MAP) to Ovsynch protocol could not mimic normal high P_4 levels required to completely prevent premature ovulation, when four cows had premature CL regression and two other cows had premature ovulation. Therefore, to ensure that animals are at the right stage of the cycle (between days 5-12, when P_4 levels are high) when Ovsynch is initiated, pre-synchronization with double injections of $PGF_{2\alpha}$ is recommended (Ambrose et al., 2000; Murugavel et al., 2003).

Alternatively, presynchronization plus progestagens can be combined to ensure the right stage of the cycle and prevention of premature estrus and ovulation. The time of AI after the second GnRH injection of the Ovsynch ovulation synchronization protocol could also influence PR. The fertile life of sperm is between 24 to 48 h and fertile life of the ovum is between 6 to 24 h (Sorensen, 1979). In this study, the cows in the Ovsynch protocol were artificially bred at 16 h after the second GnRH injection. Ovulation in lactating cows occurs between 24 and 32 of the second GnRH injection (Pursley et al., 1995). Therefore, if the sperm life span is only 24 h and ovulation occurs at 48 h, fertilization would not occur; however, if the life span of the sperm is 48 h and ovulation occurs at 48 h after the second GnRH injection, fertilization may occur. A study undertaken in our laboratory (Hirad et al., 1999) compared the effectiveness of Ovsynch/TAI on PR with double injections of $PGF_{2\alpha}$/TAI for planned breeding in dairy cows. Timed inseminations were performed at 12 and 36 hours after the second injection

of GnRH in the Ovsynch group, while in the $PGF_{2\alpha}$ group, TAI were done at 60 and 84 h after the second injection of $PGF_{2\alpha}$. The PR was significantly higher for cows in the Ovsynch group (62% vs. 43%). Therefore, two doses of AI 24 h apart following synchronization with the Ovsynch protocol may cover the anticipated window of ovulation (at least the 48 h) and improve PR following TAI.

With regard to the CIDR protocol, the first 5 mg of E_2 ought to be administered with 100 mg P_4 at the start of treatment to induce regression of the large follicle and initiate a new follicular wave 3-5 days later. The second dose of E_2, however, may need to be reduced to minimize the chance of luteolysis and termination of pregnancy. Alternatively, 0.5 mg of estradiol cypionate (ECP) can be used to replace E_2, especially since this is the only form of estrogen that is approved for use in cattle in North America (Ambrose et al., 2001; Colazo et al., 2003). Another alternative is the use of GnRH to replace E_2, because of its industry approval and common use in clinical applications.

A cost benefit analyses must be conducted by cattle producers before any decision is made on which type of synchronization protocol to be used in cattle operations. Average costs of the drugs used in this study were: \$4.5 per dose of $PGF_{2\alpha}$ (lutalyse, 25 mg); \$3.2 per dose of GnRH (Factrel®, 2 ml); \$0.3 per dose of P_4 (100 mg in 10 ml oil); \$0.5 per dose of E_2 (5 mg in 2 ml oil); \$12.0 per CIDR device; and average cost per straw of semen is \$35.0. At the UBC Dairy Education and Research Centre in Agassiz, herd persons are paid approximately \$20.0 per hour and detection of estrus occurs three times a day for 15 minutes each time. The time associated with sorting, handling, and locking

cows is approximately 15 minutes. These costs combining with the fact that up to 7-day is required for estrus detection following administration of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) 11 to 14 days apart (Lucy et al., 1986; Stephens and Rajamahendran, 1998) results in the cost per cow ranging between $64 and 144. The Ovsynch and CIDR ovulation synchronization protocols, both rely on the use of fixed TAI and elimination of estrus detection. However, the Ovsynch protocol is estimated to cost $65.9 per cow for first service conception, whereas, the CIDR protocol can cost $73.3 per cow for first service conception. The cost of the CIDR protocol may be lessened by using ECP, which can be used to replace the usage of E_2 in the CIDR protocol. Moreover, the use of CIDR is currently not permitted for use in lactating dairy cows.

6.2. Summary and conclusions

Three experiments were conducted to understand LH responses, CL formation and function, ovarian follicular dynamics, and PR following Ovsynch and CIDR ovulation synchronization/TAI protocols. The objectives of the first experiment conducted in dairy heifers were to compare LH profiles, follicular dynamics, P_4 concentrations and PR following Ovsynch and CIDR ovulation synchronization/TAI protocols at random stages of the estrous cycle. The study demonstrated that low ovulation rate in response to the first gonadotropin releasing hormone (GnRH) injection in the Ovsynch protocol did not influence ovulation synchronization rate following the second GnRH injection. However, initiation of the Ovsynch protocol late in the estrous cycle appears to cause lower ovulation synchronization rates as well as PR as a result of premature ovulation. The study also illustrated that the diameter of the ovulatory DF, and

duration of dominance of the ovulatory follicle had no effect on subsequent LH profiles, follicular dynamics, P_4 concentration, or PR between ovulation synchronization protocols. Further, the results imply that the incidences of premature ovulation and lower PR in heifers following the Ovsynch synchronization protocol could have resulted from a premature LH surge prior to the second GnRH injection, which could have adversely affected the fertility rate. It was found that the use of the CIDR protocol consistently results in high ovulation synchronization as well as PR in heifers. In the second experiment, the objective was to compare in vitro corpus luteum (CL) P_4 production following Ovsynch and CIDR ovulation synchronization protocols. Corpora lutea formed after Ovsynch and CIDR ovulation synchronization protocols yielded similar in vitro P_4 concentrations at different stages of the synchronized cycle. The response to LH, $PGF_{2\alpha}$, and LH + $PGF_{2\alpha}$ was also not different between the two synchronization protocols. In the third experiment, the objective was to conduct a large scale study in dairy cows and compare in vivo P_4 production as well as PR between cows synchronized with the Ovsynch and CIDR ovulation synchronization/TAI protocols. These data demonstrated that in both the Ovsynch and CIDR ovulation synchronization/TAI protocols, PR were substantially higher in the animals, which had synchronized ovulations. The data also demonstrated that the CIDR ovulation synchronization protocol resulted in higher PR in multiparous cows relative to the Ovsynch synchronization protocols. In vivo P_4 production was similar between the Ovsynch and CIDR ovulation synchronization/TAI protocols. However, cows treated with the CIDR treatment protocol had lower apparent embryonic mortality between days 21 and 35 post AI, when compared to the Ovsynch treatment protocol.

The present study, based on similar LH profiles, follicular dynamics, CL function and PR (both in cows and heifers) following the Ovsynch and CIDR ovulation synchronization/TAI protocols, suggests that both treatment protocols are equally effective for ovulation synchronization/TAI programs in cattle. The Ovsynch treatment protocol cost less and requires less handling than the CIDR ovulation synchronization/TAI protocol. Furthermore, the use of GnRH in the Ovsynch protocol has industry approval and is commonly used in clinical applications. Therefore, the use of Ovsynch ovulation synchronization/TAI protocol is recommended for use in cattle to eliminate estrus detection and enhance PR and success of AI programs.

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