USE OF STEROID HORMONES OR GnRH TO SYNCHRONIZE AND RESYNCHRONIZE FOLLICULAR WAVE EMERGENCE, ESTRUS, AND OVULATION IN CATTLE

A Thesis Submitted to the College of

Graduate Studies and Research

In Partial Fulfillment of the Requirements for

the Degree of Doctor of Philosophy in the

Department of Large Animal Clinical Sciences

University of Saskatchewan

Saskatoon

By

Marcos German Colazo

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ABSTRACT

A series of experiments were designed to study alternative estrus synchronization and resynchronization protocols to facilitate the use of artificial insemination in cattle.

Studies were conducted to study the effects of estradiol cypionate (ECP) on follicular dynamics, time of ovulation, and pregnancy rate to timed-AI (TAI) in CIDR-based protocols. Although administration of 1 mg ECP did not result in synchronous follicular wave emergence, a dose of 0.5 mg ECP synchronized LH release and ovulation. Administration of ECP 24 h after CIDR removal resulted in acceptable pregnancy rate. However, treatment with ECP at CIDR removal resulted in acceptable pregnancy rate only if follicular wave emergence was synchronized with estradiol-17β (E-17β).

The efficacy of two estradiol preparations (5 mg of E-17β or estadiol valerate; EV) and reduced doses of EV on CL and ovarian follicular dynamics and superovulatory response were examined. When doses of 5 mg were compared, EV treatment resulted in a more variable interval to follicular wave emergence and a lower superovulatory response than E-17β. However, EV at a dose of 1 or 2 mg was efficacious in synchronizing follicle wave emergence in CIDR-treated cattle.

Pregnancy rates were compared following TAI in cattle given a new or previously used CIDR and injections of estradiol, with or without progesterone, to synchronize follicular wave emergence. Pregnancy rate following TAI did not differ between cattle treated with a new or once-used CIDR, but pregnancy rate was lower in cattle treated

with one or two twice-used CIDR. The addition of an injection of progesterone to the estradiol treatment at CIDR insertion did not enhance pregnancy rate

The efficacy of progestins (used CIDR and MGA), and E-17β, ECP, GnRH, or progesterone treatment for resynchronization of estrus in cattle not pregnant following TAI were investigated. Progestin treatment resulted in the majority of nonpregnant heifers detected in estrus over a 4-d interval. Conception rates were higher in heifers resynchronized with a once-used CIDR than with MGA. GnRH at CIDR insertion synchronized follicular wave emergence in cows, but did not increase conception rate in heifers. E-17β at CIDR insertion (1.5 mg) and removal (0.5 mg) resulted in decreased pregnancy rate following TAI. In summary, protocols described in this thesis resulted in acceptable pregnancy rates following TAI and resynchronization of previously inseminated heifers with progestins resulted in variable estrus and pregnancy rates.

ACKNOWLEDGEMENTS

I wish to express my appreciation to my mentor Dr. Reuben J. Mapletoft, who has provided encouragement, patience, and guidance throughout my PhD program at WCVM. I sincerely thank my co-supervisor, Dr. John Kastelic for his valuable advice, technical assistance, and also for his constructive criticisms of my manuscripts.

I would like to acknowledge my committee members for their time, effort, and guidance: Dr. Terry Carruthers, Dr. Roger Pierson, and Dr. Gregg Adams.

I am so grateful to the University of Saskatchewan, AAFC, Brandon and Lethbridge Research Centers, and the cooperating producers for providing financial support, animals and equipment for my studies. I am especially indebted to Dr. Julie Small, Dr. Divakar Ambrose, Mr. Douglas Ward, the beef crew from AAFC, Brandon Research Centre, Mr. Randy Wilde, Mr. William Kerr and the staff from the University of Saskatchewan Goodale farm, Mr. Bernie Loman, Mr. Ken and Mrs. Sharon Piller, Mr. Morley Forsyth and family, and summer students Dr. Patrick Witthaker, Mr. Quinn Gavaga, Mr. Nathan Erickson, Ms. Mary Rutledge, and Mr. Lucas Siqueira for their assistance.

I would like also thank my fellow graduate students, colleagues and friends that I had the opportunity to know in Saskatoon: Dr. Marcelo Martínez, Andres Arteaga and family, Andrzej Palasz, Derek Haley, Leonardo Brito and family, Rajesh Jaiswal, Robert McCorkell, Pritpal Malhi, Saravanan Durairaj, Christy Barlund, Tal Raz, Sameeh

Abutarbush, Marcelo Ratto and family, Dr. Manuel Chirino-Trejo and family, Dr. Jorge Chedrese and family, Mr. Murray Dyck and family, Mr. Nick Prpich and family and Dr. Mapletoft's family for the support they have provided me over the last six years.

I express my gratitude to the professors who have increased my knowledge, Dr. Albert Barth, Dr. Jaswant Singh, Dr. Norman Rawlings, Dr. Peter Flood, Dr. Robert Baker, and Dr. Cheryl Waldner for statistical advice and the staff from Department of Large Animal Clinical Sciences, especially Ms. Tracey Gilles and Mrs. Jackie Bahnmann for helping me to solve many administrative problems.

Finally, I want to thank my parents Hector and Sofia, and my sisters Analia, Cecilia and their families, my family in law Hugo, Maria Susana and Elvira for the spiritual support in not only these six years but also in all my career. Finally, but certainly not the least, I want to thank my family, my wife, Maria Daniela, my daughter, Camila and my son, Juan Manuel. May God bless each of you!!!!

DEDICATION

I dedicate this thesis to my family, my wife, Maria Daniela, my daughter, Camila and my son, Juan Manuel for supporting me with their love. I also dedicate this thesis to my uncle Juan Carlos, who encouraged me to do research and passed away during my PhD program.

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LIST OF ABREVIATIONS

AI	artificial insemination
ANOVA	analysis of variance
3β-HSD	3beta-hydroxysteroid dehydrogenase
BMP	bone morphogenetic proteins
CIDR	controlled internal drug release
CL	corpus luteum
COX-2	cyclooxygenase-2
CP	commercial progesterone
d	days
DHA	docosahexaenoic
EB	estradiol benzoate
EB24	estradiol benzoate 24 h after CIDR removal
ECP	estradiol cypionate
ECP0	estradiol cypionate at CIDR removal
ECP24	estradiol cypionate 24 h after CIDR removal
EGF	epidermal growth factor
EN	estradiol valerate and norgestomet
EP	estradiol-17β and progesterone
EPA	eicosapentaenoic
E-17β	estradiol-17β
EV	estradiol valerate

FAME	fatty acid methyl esters
FGF	fibroblast growth factor
FSH	follicle stimulating hormone
FWE	follicular wave emergence
g	grams
GDF	growth differentiation factor
GEE	General Estimating Equations
GnRH	gonadotrophin releasing hormone
GnSAF	gonadotrophin surge-attenuating factor
GnRH52	gonadotrophin releasing hormone at 52 h after CIDR removal
h	hours
hCG	human chorionic gonadotrophin
IGFBP	IGF-bindings proteins
IGF	insulin-like growth factor
im	intramuscular
INF-τ	interferon-tau
IU	international units
kg	kilogram
Km	kilometer
L	lactating
LH	luteinizing hormone
LLC	large luteal cells
LSD	least significant difference

mg	milligram
MGA	melengestrol acetate
MHz	megahertz
mL	milliliter
mm	millimeter
min	minute
mo	months
mRNA	messenger ribonucleic acid
ng	nanogram
NAHMS	National Animal Health Monitoring Systems
NL	non-lactating
P	progesterone
P450scc	cytochrome P450 side chain cleavage
P450c17	cytochrome P450 17alpha-hydroxylase
P450arom	cytochrome P450 aromatase
pg	picogram
PGF	prostaglandin $F_{2\alpha}$
pLH	porcine luteinizing hormone
±	plus/minus
PUFA	polyunsaturated fatty acids
SAS	Statistic Analysis Software
SD	standard deviation
SEM	standard error of the mean

SMB™	Syncro-Mate B
SPSS	Statistical Package for the Social Sciences
StAR	steroidogenic acute regulatory protein
TAI	timed-AI
TGF-β	transforming growing factor-β
µg	microgram
µmm	micromillimeter
VEGF	vascular endothelial growth factor

1.0 GENERAL INTRODUCTION

Our knowledge of the physiology and endocrinology of reproduction in domestic animals has increased noticeably over the past few years. This has led to a tremendous increase in the development of biotechnological procedures for the control of farm animal reproduction. Several methods for synchronizing the estrous cycles of cattle, and other farm animals have been developed. Embryo transfer procedures have been improved; embryos are now produced by fertilization in vivo or in vitro. Further, sexing of embryos and methods for separating X- and Y-bearing sperm are available. Transgenic and cloned farm animal offspring have been produced by nuclear transfer techniques. These biotechnological procedures, along with intense selection to identify genetically superior females and males could result in a great impact on the genetic improvement of dairy and beef herds. However, the beef industry, in particular, has been slow to adopt these technological advances and none are currently having much impact on animal agriculture worldwide.

Artificial insemination (AI) has been widely used for breeding dairy cattle, and has become one of the most important techniques for the genetic improvement of farm animals. Almost 90% of dairy producers in North America use AI as a method to breed cattle, compared to only 5% of beef producers (NAHMS, 1997). One of the main reasons for the low adoption of AI in the beef industry is that estrus detection is required. Estrus

detection is labour and time consuming, especially in large beef herds in extensive management. In addition, the efficiency of estrus detection has been reported to be ≤ 50% in dairy herds across North America. Poor estrus detection is associated with low pregnancy rates following AI, which in turn is the principal factor limiting cattle productivity and profitability. Therefore, improvements in the synchronization of estrus and ovulation, allowing for timed-AI (TAI) with a high success rate, will enable heifers and cows to be artificially bred to genetically superior sires, and lead to optimal calving intervals and greater profits for cattle producers.

2.0 REVIEW OF LITERATURE

2.1 Overview of the estrous cycle

Antral follicle growth during the estrous cycle in cattle occurs in wave-like patterns (Rajakoski 1960; Pierson and Ginther, 1984; Pierson and Ginther, 1987; Savio et al., 1988; Sirois and Fortune, 1988; Ginther et al., 1989a; 1989b; Knopf et al., 1991). Most cattle have been shown to have two or three follicular waves per cycle. The proportions of animals with two or three follicular waves have varied among reports (Ginther et al., 1989b; Sirois and Fortune, 1988; Savio et al., 1990; 1993). The first follicular wave typically emerges on the day of ovulation (Day 0) with no difference between two or three follicular wave cycles. The second follicular wave emerges 1 or 2 d earlier in three-wave cycles (Day 8, 9 or 10) than in two-wave cycles, and in three-wave cycles, a third follicular wave emerges on Day 15 or 16. It is not clear why some cows have two follicular waves per cycle while others have three waves; however, it has been shown that follicular wave pattern may be affected by the energy level in the ration (Murphy et al., 1991) and may change from one estrous cycle to the next (Price and Carrière, 2004).

Two recent studies suggested that conception rate to first service was reduced in beef and dairy cattle in which the ovulatory follicle came from the second compared to the third follicular wave during the estrous cycle (Ahmad et al., 1997; Townson et al., 2002). In another study, pregnancy rate did not differ among lactating dairy cows with different wave patterns of follicle development, although interval from follicular wave emergence to estrus was negatively related to fertility (Bleach et al., 2004).

Follicular wave emergence has been defined as synchronous growth (within 1 to 2 d) of 8 to 41 small follicles (3 to 5 mm in diameter; Ginther et al., 1989a, 1996a). Within 3 d of initiation of a wave, one follicle is selected as the dominant follicle. This follicle continues to grow and the others in its cohort (subordinates) regress; this event is called deviation or selection (Ginther et al., 1996a). The time of deviation is further defined as the beginning of the greatest difference between the two largest follicles in a wave; this occurs on average 2.8 d after emergence, when the future dominant follicle was 8.5 mm in diameter. The dominant follicle eventually regresses or ovulates depending on the endocrine conditions during its tenure of dominance.

During the late luteal phase (Day 16 to 18 of the estrous cycle), prostaglandin $F_{2\alpha}$ (PGF) is released in a pulsatile fashion from the nongravid uterus and passes through a local utero-ovarian vascular pathway to the ovary and causes luteal regression (Ginther, 1974). The dominant follicle present at the onset of luteolysis will become the ovulatory follicle, and emergence of the next follicular wave is delayed until the day of the ensuing ovulation.

Interovulatory intervals in two-wave cycles have been shown to be shorter than those of three-wave cycles (20.4 ± 0.3 versus 22.8 ± 0.6 d; Ginther et al., 1989b). Hence, the 21-d estrous cycle in cattle is rarely found in nature; it is an average between two- and three-wave cycle animals.

2.2 Endocrine regulation of antral follicles

2.2.1 *Growth pattern of ovarian antral follicle development*

The growth pattern of ovarian antral follicle development has been arbitrarily divided in two distinct phases (Mihm and Bleach, 2003). A 'slow' growth phase from acquisition of an antrum to a size detectable by ultrasonographic examinations (3 to 5 mm) and the 'fast' growth phase from the time of follicular wave emergence to atresia or ovulation of the dominant follicle. Growth of ovarian antral follicles from acquisition of an antrum (0.3 mm) to 3 to 5 mm in diameter ('slow' phase) takes more than 30 d (Lussier et al., 1987). In cattle, it is still unclear whether this stage of follicle development is possible without follicle stimulating hormone (FSH). However, there is evidence that FSH receptors are present (Xu et al., 1995; Bao and Garverick, 1998) and functionally active (McNatty et al., 1999) during preantral and early antral development in the bovine species, suggesting that FSH may have a role in the early stages of follicular development. In addition, murine follicles that are unable to respond to FSH do not progress from preantral stages to early antral stages of development (Abel et al., 2000). Conversely, it is well known that follicles in the 'fast' phase are absolutely

dependent on adequate concentrations of both FSH and luteinizing hormone (LH; reviewed in Mihm and Bleach, 2003).

2.2.2 *Recruitment of follicular waves*

As mentioned earlier, follicular wave emergence has been defined as synchronous growth of several small follicles (3 to 5 mm of diameter). The emergence of each follicular wave is preceded by a transient increase in FSH in cycling cattle (Adams et al., 1992a; Sunderland et al., 1994; Gong et al., 1995; Bodensteiner et al., 1996), in calves (Evans et al., 1994; Adams et al., 1994), during pregnancy, and in the postpartum anestrous period (Ginther et al., 1996b). Suppression of the FSH increase, with either bovine follicular fluid (Turzillo and Fortune, 1993) or exogenous estradiol (Bó et al., 1995a,b) prevents new wave emergence, while follicular wave emergence will occur when FSH is concurrently administrated (Bergfelt et al., 1994a). On average, all follicles in a wave (FSH-dependent) grow at a similar rate and produce considerable amounts of systemic FSH suppressants (e.g. inhibin and estradiol) that are responsible for the decline of FSH to basal levels. When FSH declines, FSH-dependent follicles begin to show characteristic follicular fluid changes leading to atresia (Mihm et al., 1997; Austin et al., 2001). Administration of physiological amounts of FSH during the FSH decline prevents characteristic changes in follicular fluid and atresia of subordinate follicles (Adams et al., 1993a; Mihm et al., 1997). Only the selected follicle (i.e. the dominant follicle) is capable of growing in a low FSH environment because it acquires LH dependency and has a competitive advantage over follicles destined to become atretic (which are still dependent

on FSH). However, basal FSH concentrations are still essential for the dominant follicle to maintain several follicular functions, such as aromatase activity, and insulin-like growth factor (IGF), activin and inhibin synthesis (Badinga et al., 1992; Mihm et al., 1997; Austin et al., 2001).

2.2.3 *Selection of follicles for dominance*

The future dominant follicle has to gain the capacity to survive during a decline in FSH by continuing to grow and produce estradiol, while granulosa cells in subordinate follicles undergo apoptosis (Austin et al., 1997). As explained earlier, the term follicular deviation or selection usually refers to the stage of the follicular wave when a perceptible difference in size is observed between the dominant follicle and its largest subordinate. However, it is likely that selection of the future dominant follicle is a progressive process and that the initial stages of selection occur even before the 'fast' growth phase of follicle development. Based on ultrasonographic studies in heifers, it has been proposed that the future dominant follicle emerges 6 to 12 h earlier than the future largest subordinate follicle (Ginther et al., 2001; Jaiswal et al., 2004).

One of the most significant characteristics of the dominant follicle is the increased production of estradiol; this is associated with a termination in the rise of FSH and with a return of FSH to basal levels (Ginther et al., 2000). Concentrations of estradiol in follicular fluid were much higher in dominant follicle isolated on Day 5 of the estrous cycle than in recruited follicles on Day 3, whereas subordinate follicles on Day 5 had

concentrations of estradiol similar to recruited follicles on Day 3 (Fortune, 1994; Badinga et al., 1992; Fortune et al., 2001). Bao and Garverick, (1998) showed that dominant follicles have higher levels of messenger ribonucleic acid (mRNA) for LH receptors (in theca and granulosa cells) and enzymes involved in androgen and progestin synthesis (cytochrome P450 side chain cleavage [P450scc], cytochrome P450 17alpha-hydroxylase [P450c17], cytochrome P450 aromatase [P450arom], 3beta-hydroxysteroid dehydrogenase [3β-HSD], and steroidogenic acute regulatory protein [StAR]), than recruited follicles. Although it is not clear why granulosa cells of dominant follicles attain LH receptors, it has been hypothesized that this allows it involves a shift from FSH- to LH-dependence and an increase in the capacity for estradiol production (Ginther et al., 2001).

Another still unknown paradigm is why only one follicle is selected to be dominant from a group of similar follicles. There is increasing evidence that the IGF system may play a critical role in selection of the dominant follicle (Fortune et al., 2001). Insulin-like growth factors sustain follicular growth by stimulating granulosa cell proliferation and synergizing with gonadotrophins to promote differentiation of both theca and granulosa cells (Spicer and Echternkamp, 1995). Therefore, follicles within the cohort with higher follicular fluid concentrations of available IGF are more likely to become dominant. Interesting, changes in components of the intrafollicular IGF system, particularly concentrations of low molecular weight IGF-binding proteins (IGFBP) seem to be closely related to selection of the future dominant follicle (Fortune et al., 2001). In this regard, Mihm et al. (2000) reported that, based on bovine follicular fluid samples

taken on Day 1.5 of the first follicular wave, the follicle with the lowest concentration of IGFBP-4 always became the dominant follicle. In addition, Armstrong et al. (1998) reported that IGFBP-2 was lower in the dominant follicle compared to subordinate follicles.

In high producing dairy cows, the mechanism of follicle deviation seems to be disrupted; the incidence of double ovulation was shown to be three times greater in high than in low producing cows (Fricke and Wiltbank, 1999). Lactating dairy cows with high milk production have increased blood flow to the liver, which increases steroid hormone metabolism (Wiltbank et al., 2000) resulting in lower levels of steroid hormones in the peripheral circulation than dairy heifers (Sartori et al., 2004; Wolfenson et al., 2004). Wiltbank et al. (2000) proposed that as a result of decreased circulating estradiol concentrations, FSH remains elevated allowing more than one follicle to become dominant. However, the possibility of involvement of additional mechanisms cannot be dismissed. In this regard, double ovulations were more frequently observed in cows with three (28.6%) than with two (1.7%) follicle waves per estrous cycle (Bleach et al., 2004), and if luteolysis was induced at the time of follicular wave emergence (Mussard et al., 2003) indicating that progesterone concentrations and LH pulsatility may also play important roles in the mechanisms of dominant follicle selection.

After the decline in circulating FSH, LH secretion seems to be critical to maintain dominant follicle viability. Treatment with exogenous progesterone during early metestrus has been shown to suppress the diameter of the first-wave dominant follicle in

a dose-dependent manner (Adams et al., 1992b; Burke et al., 1994), possibly by suppressing LH pulse frequency (Ireland and Roche, 1982; Goodman and Karsch, 1980; Price and Webb, 1988). Administration of LH pulses prolongs the dominance of the first wave dominant follicle in cyclic cows (Taft et al., 1996) and causes increased estradiol secretion in postpartum anestrous beef cows (Duffy et al., 2000). Moreover, low progesterone concentrations in cattle treated with progestagens resulted in increased LH pulse frequency, which in turn prolonged the dominance of the dominant follicles destined to ovulate (Savio et al., 1993; Stock and Fortune, 1993).

Continued suppression of LH (as a consequence of luteal-phase progesterone secretion) causes the dominant follicle to cease its metabolic functions and eventually it undergoes atresia. Soon after the decrease in its metabolic functions, there is an increase in circulating FSH concentrations, and the emergence of a new follicular wave, with the ovarian follicle cycle repeating itself. However, at the end of the luteal phase, a reduction in circulating progesterone concentration results in increased LH pulse frequency and increased estradiol secretion, which are responsible for the preovulatory surge of gonadotrophins and ovulation of the preovulatory follicle (Nett et al., 1984; Bryner et al., 1990; Nett et al., 2002).

2.3 Estrus synchronization

Effective synchronization of estrus in beef and dairy cattle has been a goal of the cattle industry since AI techniques first became available. In the last 60 years, many hormonal or technical approaches have been evaluated to minimize the need for estrus detection in cattle. Prostaglandin $F_{2\alpha}$ and its analogues are the most common treatment used to shorten the luteal phase and synchronize estrus, thereby reducing the need for estrus detection. Progestagens have been used to extend the luteal phase, resulting in synchronous estrus after progestogen withdrawal. More recently, gonadotrophin releasing hormone (GnRH) and estradiol alone or in combination with progesterone have been used in estrus synchronization programs. These will be described in control of follicular wave patterns.

2.3.1 *Estrus synchronization with prostaglandin*

The effectiveness of PGF in causing luteolysis and inducing estrus and ovulation in cattle is well established. The luteolytic properties of exogenous PGF in cattle have been reported since 1972 (see Seguin 1980). Three important factors regarding PGF as a treatment for estrus synchronization must be considered. Firstly, it is necessary that a functional corpus luteum (CL) be present for PGF to be effective. Early postpartum anestrous cows and prepuberal heifers are not good candidates for PGF protocols because they will not have a functional CL. Secondly, the CL prior to Day 5 after ovulation is nonresponsive to a single injection of PGF (Momont and Seguin, 1982; Wiltbank, 1997).

The unresponsiveness of the early CL is apparently not due to a lack of PGF receptors; early and late CL were not different with respect to numbers and affinity of PGF receptors (Sakamoto et al., 1994; 1995; Wiltbank et al., 1995). Although responsiveness increases progressively from Day 6 until Day 8 of the estrous cycle (Momont and Seguin, 1982; 1984; Kastelic et al., 1990a), there is a difference, in term of early CL response to PGF treatment, between heifers and cows (Wiltbank et al., 1997). The CL in cows has been reported to be unresponsive until Day 6-7 post-estrus, whereas the CL in heifers was responsive on Day 5 (Wiltbank et al., 1997). To overcome this problem, a double-injection system of two doses of PGF separated by 11 (in heifers) to 14 d (in cows) is usually used to ensure that a high proportion of animals have a responsive CL at the time of the second PGF treatment. Although the double dose approach reduces estrus detection, it may result in reduced conception rates, particularly in those animals responding to the first PGF treatment (Xu et al., 1997). Low progesterone concentrations prior to the second PGF treatment seems to account for the reduced fertility since the addition of a CIDR insert restored fertility (Xu et al., 1997). The third factor to take into account when using PGF, is the great variability in the interval from treatment to behavioral estrus and ovulation among treated animals. In the presence of a responsive CL, estrus can be induced by a single administration of PGF; however, the interval to the resulting estrus and ovulation is dependent on the stage of development of the dominant follicle at the time of treatment (Kastelic et al., 1990a; Kastelic and Ginther, 1991).

2.3.2 *Estrus synchronization with progestagens*

Since the discovery that progesterone inhibits estrus and ovulation (Christian and Casida, 1948; Ulberg et al., 1951), administration of progesterone or progestagens have used in synchronization schemes to improve reproductive management (Trimberger and Hansel, 1955; Hansel et al., 1961; Wiltbank et al., 1967). Relatively long durations of progestogen treatment (i.e., ≥ 14 d), which allowed for spontaneous occurrence of luteolysis before treatment withdrawal, resulted in synchronous estrus, but reduced fertility (Trimberger and Hansel, 1955; Hansel et al., 1961; Wiltbank et al., 1967). However, it was not realized until the use of transrectal ultrasonography and endocrine analysis (approximately 30 years later) why fertility is compromised in cattle with spontaneous luteal regression prior to progestagen withdrawal (Sirois and Fortune, 1990; Savio et al., 1993; Stock and Fortune, 1993). Essentially all commercially available progestagen treatments do not produce progesterone/progestagen concentrations that are comparable to that during luteal phase. Although 'low' progestagen concentrations will inhibit behavioural estrus and the preovulatory LH surge, they are insufficient to inhibit normal pulsatile LH secretion, which will stimulate continued follicular growth. Follicles with prolonged growth profiles ("persistent follicles") have been extensively described in the literature (Sirois and Fortune, 1990; Savio et al., 1993; Stock and Fortune, 1993; Revah and Butler, 1996; Kinder et al., 1996; Kojima et al., 2003).

Cattle that ovulated a persistent follicle had lower pregnancy rates than those that ovulated a "healthy" dominant follicle (Savio et al., 1992; Stock and Fortune, 1993; Mihm et al., 1994). Although fertilization rate and mean number of accessory sperm in the zona pellucida was not affected in beef cows that ovulated a persistent follicle, fewer embryos reached ≥ 16-cell stage of development (Ahmad et al., 1995). Death of embryos in cattle ovulating a persistent follicle may be due to premature activation of the oocyte (Revah and Buttler, 1996; Mihm et al., 1999), or high and prolonged exposure to estrogen, which could alter the pattern of oviductal secretory proteins (Binelli et al., 1999). The uterine environment is unlikely to be inappropriate since pregnancy rate following transfer of embryos 7 d after estrus did not differ from cows that ovulated a normal dominant follicle (Wehrman et al., 1996b).

Before widespread use of ultrasonography, several scientists attempted to improve the fertility of progestagen-treated animals by shortening the duration of progestagen treatment. Pharmacological doses of estradiol (Wiltbank and Kasson, 1968; Mauleon, 1974) or PGF (Wishart, 1974; Thimonier et al., 1975) were given in combination with 'short' progestagen treatment to induce luteolysis. Estrus synchrony and fertility responses improved, but remained quite variable (Wishart, 1974; Thimonier et al., 1975).

In summary, estrus synchronization schemes involving the use of PGF, progestagens or combinations of both fairly predictably induce luteolysis and are able to induce estrus at a desired time, however, variability in interval to estrus and fertility remains an impediment to their successful use. Moreover, all these schemes still require

that estrus detection be performed. Hence, alternative approaches to controlling ovarian follicle growth must be undertaken to overcome the problems associated with the development of persistent follicles or the occurrence of asynchronous estrus and ovulation.

2.4 Control of ovarian follicle dynamics during diestrus

2.4.1. *Removal of the suppressive effect of the dominant follicle: Physical methods of removal*

Physical methods of removing the suppressive effect of the dominant follicle have included electrocautery and transvaginal ultrasound-guided follicle ablation. Cauterization of the dominant follicle of heifers on Days 3 or 5 (day of ovulation = Day 0) resulted in follicular wave emergence in 2.5 ± 0.4 and 2.0 ± 0.3 d after cauterization, respectively (Ko et al., 1991). Dominant follicle cauterization caused early emergence of wave 2 and increased the incidence of 3-wave interovulatory intervals when compared to controls. Similarly, Adams et al. (1993b) found that the emergence of wave 2 was hastened (Day 6.3 versus 9.3) and more heifers had three waves per interovulatory interval (5/6 versus 2/7) when the dominant follicle was cauterized on Day 3 of the estrous cycle.

Transvaginal ultrasound-guided follicle ablation for the synchronization of follicular wave emergence was first reported by Bergfelt et al. (1994b). Ablation of all follicles ≥ 5 mm in diameter at random stages of the estrous cycle in heifers resulted in a transient increase in FSH and synchronous emergence of a new follicular wave approximately 2 d after ablation. Subsequent experiments demonstrated that FSH treatments given 1 d following follicle ablation resulted in superovulatory responses that were similar to that achieved when FSH was given at the expected time of emergence of the second follicular wave (Bergfelt et al., 1997) or hormonal-induced follicular wave emergence (Baracaldo et al., 2000). Thus, transvaginal ultrasound-guided follicle aspiration combined with PGF and LH or GnRH treatment was successfully used to synchronize ovulation in cattle (Bergfelt et al., 1994b; Brogliatti et al., 1998). Although physical methods of follicle ablation resulted in a very precise control over the initiation of a new follicular wave, they are not very practical approaches to implement in the field.

2.4.2 *Gonadotrophin releasing hormone (GnRH)*

Administration of GnRH produces acute increases in LH and FSH (Kaltenbach et al., 1974; Chenault et al., 1990; Martínez et al., 2003b), with the magnitude depending on stage of the follicular wave (Kastelic and Mapletoft, 1998). The LH surge is induced, on average 2 h after GnRH treatment (Chenault et al., 1990; Martínez et al., 2003b), which in turn will induce ovulation in those animals that have a dominant follicle ≥ 9 mm in diameter (Martínez et al., 1999). It was also proposed that either ovulation or luteinization of a dominant follicle after GnRH treatment was followed by emergence of

a new follicular wave (Macmillan and Thatcher, 1991). However, new wave emergence, which usually occurs within 2 d (Twagiramungu et al., 1995b), was synchronous only if ovulation of the dominant follicle was induced (Martínez et al., 1999). Since follicular waves emerge approximately every 7 to 10 d (see overview of the estrous cycle), spontaneous emergence of a new wave (in the absence of ovulation) could occur at approximately the same time as the emergence of a new wave following GnRH-induced ovulation in some heifers (Martínez et al., 1999). Although the occurrence of luteinization of follicular wall cells following GnRH administration in cyclic animals has not been adequately studied, luteinization has been observed following treatment of follicular cysts with GnRH (Kesler et al., 1981).

Several factors may determine whether ovulation occurs in response to GnRH treatment. The most important is likely to be the day of the estrous cycle (Martínez et al., 1999; Moreira et al., 2000) i.e., progesterone concentrations and the size of the dominant follicle at the time of GnRH administration. The former is likely to affect the magnitude of LH release (Di Gregorio and Nett, 1995) and the latter is related to attainment of LH receptors on granulosa cells (Xu et al., 1995). Although ovulatory response to an injection of GnRH was higher on Days 5 and 15 to 18 of the estrous cycle (dominant follicle ≥ 10 mm; Moreira et al., 2000), ovulation did not occur consistently in heifers expected to have a dominant follicle > 10 mm diameter at the time of treatment (Martínez et al., 2003b). In addition, Pursley et al. (1995) reported that ovulation occurred in approximately 55.0% of GnRH-treated heifers versus 85.0% of GnRH-treated lactating dairy cows, regardless of the stage of the estrous cycle. Hence, following GnRH

treatment, ovarian follicle development is not effectively controlled in heifers as compared to cows. In addition, dose (Mihm et al., 1998) and commercial preparation (Martínez et al., 2003b) have been reported to affect the ovulatory response to GnRH treatment during diestrus in cattle.

2.4.3 *Luteinizing hormone (LH)*

Because progesterone has a negative effect on pituitary LH release, exogenous LH administration during diestrus is expected to result in a greater ovulatory response than GnRH administration. Treatment of beef heifers with porcine LH (pLH) at precise stages of the first follicular wave (Days 3, 6, or 9 after spontaneous ovulation) resulted in ovulation in 78% of heifers (Martínez et al., 1999), which was higher than following the administration of GnRH in the same study (56%) or that reported by Pursley et al. (1995) in dairy heifers. Although this finding would suggest that LH might be more effective in synchronizing follicular wave emergence in heifers than GnRH, LH has not been widely used. The main reason may be cost, because the price of pLH (Lutrophin-V) is double that of commercially available GnRH preparations, even at half of the recommended dose of 25 mg Armour. One study investigated the ovulatory response to different doses of pLH to induce ovulation of a dominant follicle in beef heifers. Oswald et al. (2000) reported that the proportion of heifers, with a dominant follicle ≥ 9 mm, that ovulated to an LH treatment was 66.6, 100, and 71.4% for doses of 5, 10, and 25 mg Armour of pLH, respectively. This result suggested that the dose of Lutropin-V could be reduced to 5 mg without significantly affecting its efficacy in inducing ovulation of a dominant follicle.

However, in another experiment, Ambrose et al. (2004) reported that none of the Holstein heifers receiving 5 mg of pLH ovulated, compared to 50% of those treated with 100 μg of GnRH. Therefore, more studies are needed to further examine the efficacy of reduced doses of pLH to control ovarian follicular dynamics.

2.4.4 *Human chorionic gonadotropin (hCG)*

Human chorionic gonadotrophin may also be used to synchronize follicular wave emergence. Ovulation and formation of an accessory CL following administration of various doses of hCG during diestrus have been reported (Price and Webb, 1989; Rajamahendran and Sianangama; 1992; Fricke et al., 1993; Sianangama and Rajamahendran, 1996; Schmitt et al., 1996a,b; Diaz et al., 1998). However, few studies have characterized ovarian follicular dynamics following hCG treatment (Sianangama and Rajamahendran, 1996; Diaz et al., 1998). Administration of hCG (1000 international units; IU) to lactating Holstein cows at the time of insemination (Day 0), Day 7, or Day 14 resulted in ovulation in 37.5, 77.7, and 44.4% of cows, respectively (Rajamahendran and Sianangama, 1992). In another experiment (Sianangama and Rajamahendran, 1996), ovulation of Day-7 follicles occurred in 100% of hCG-treated cows, and the time of emergence of the second wave of follicular growth was advanced compared to that of control cows (10.8 ± 0.3 versus 12.7 ± 1.4 d). However, the second-wave dominant follicle had a slower growth rate in cows treated with hCG compared with that of the controls (0.8 versus 1.3 mm/d). It is also noteworthy that the second-wave dominant

follicle was the ovulatory follicle in 83.3% of control cows, but only in 50% of hCG-treated cows.

Recently, Diaz et al. (1998) treated heifers on Day 5 of the estrous cycle with 3000 IU of hCG. All treated-heifers formed an accessory CL and had three follicular waves. The second wave emerged earlier and maximum diameter of the dominant follicle was smaller in hCG-treated heifers than in control heifers (7.3 versus 10.4 d and 12.8 ± 0.8 versus 15.6 ± 0.8 mm). However, the interovulatory interval did not differ between hCG and control groups (22.9 ± 0.9 versus 22.1 ± 0.9 d). Although ovulatory response and day of follicular wave emergence following hCG treatment appears to be similar to that reported for GnRH or pLH treatments, hCG has not been widely incorporated into estrus synchronization protocols. Rather, hCG has been used to improve fertility following AI or embryo transfer because of its ability to increase plasma progesterone concentrations.

2.4.5 *Steroid hormones: Progesterone & estradiol*

As described earlier, the administration of exogenous progesterone reduced the size of the dominant follicle of the first follicular wave (Adams et al., 1992; Burke et al., 1994) by reducing LH pulses (Ireland and Roche, 1982; Goodman and Karsch, 1980; Price and Webb, 1988). Suppression of LH pulses for 30 h seemed to be enough to induce atresia of a persistent follicle and this was achieved with treatment with a progesterone insert containing 1.9 g of progesterone for 24 h (McDowell et al., 1998;

Cavalieri et al., 1998). In other studies, atresia of persistent follicles was attained with a single injection of either 100 mg progesterone in oil in *Bos indicus* (Cavallieri et al., 1998) or 200 mg progesterone in oil in *Bos taurus* (Anderson and Day, 1994) cattle. Injection of 200 mg progesterone in cattle with persistent follicles resulted in emergence of a new follicular wave 3.5 ± 0.3 d later (Anderson and Day, 1994). Although, a single injection of progesterone has provided a practical and useful approach to the synchronization of follicular wave emergence in cattle with persistent follicle, it may not be reliable in other physiological conditions. Progesterone administration suppressed LH pulse frequency (Ireland and Roche, 1982) but had no significant effect on FSH secretion (Goodman and Karsch, 1980; Price and Webb, 1988; Adams et al., 1992), so it is potently capable of inducing atresia of only LH dependent follicle. Therefore, administration of physiological concentrations of progesterone is unlikely to be useful in the synchronization of follicular wave emergence if atresia of both FSH- and LH-dependent follicles is intended (i.e when treatments are given at random stages of the estrous cycle).

Administration of exogenous estradiol to nonlactating Holstein cows on Day 16 of the estrous cycle induced atresia of the potential preovulatory follicle (Engelhardt et al., 1989). However, administration of estradiol alone in heifers on Day 1 of the estrous cycle resulted in transient or incomplete suppression of the dominant follicle and delayed emergence of the next follicular wave (Bó et al., 1994). In the absence of progesterone, bolus administration of estradiol, which results in superphysiological concentrations in the circulation, initially inhibited secretion of gonadotrophins for several hours, and this was followed by a surge release of LH and FSH (Kesner et al 1981; Butler el al., 1983;

Martínez et al., 2003a). However, physiological concentrations or administration of exogenous estradiol via sequential implants in sheep and cattle with low progesterone concentration, has been reported to have variable effects on LH secretion. The administration of estradiol decreased LH pulse amplitude in sheep (Goodman and Karsch, 1980; Rawlings et al., 1984) and cattle (Price and Webb, 1988), but did not significantly suppress LH pulse frequency (Goodman and Karsch, 1980; Price and Webb, 1988). In a series of experiments using the ovariectomized bovine model, estradiol consistently increased mean concentration and pulse amplitude of LH (Day et al., 1986; Imakawa et al., 1986; Stumpf et al., 1988). However, estradiol treatment resulted in decreased (Day et al., 1986; Imakawa et al., 1986) or no effect (Stumpf et al., 1988) on LH pulse frequency. Conversely, suppression of FSH secretion has been shown to occur after treatment with an injection or implant of estradiol in ovariectomized (Butler et al., 1983; Bolt et al., 1990; O'Rourke et al., 2000; Martínez et al., 2003a) or intact (Price and Webb, 1988; Bó et al., 1994) cattle. As indicated earlier, estradiol-induced gonadotrophin suppression was followed in approximately 6 h by a dramatic increase in LH concentration in both ovariectomized and non-progesterone implanted animals, indicating that the differential effect of estradiol on gonadotrophin hormones would depend on progesterone concentrations at the time of treatment. In fact, the FSH suppression following treatment with estradiol was more prolonged in progestagen-implanted heifers whether they had been ovariectomized (Bolt et al., 1990) or not (Bó et al., 1994). In addition, several studies have suggested that estradiol and progesterone given in combination have an additive suppressive effect on LH secretion (Goodman and Karsch, 1980; Price and Webb, 1988; Stumpf et al., 1993; Bó et al., 1994). Collectively, these

studies suggest that a more controlled gonadotrophin secretion and subsequently more precise manipulation of ovarian follicular dynamics could be attained if both steroid hormones were given in combination.

The combination of estradiol and progesterone treatment to control follicular dynamics has been extensively studied in the past 10 years. Bó et al. (1995b) demonstrated that administration of 5 mg of estradiol-17β (E-17β) to progestagen-implanted heifers will induce follicular regression and emergence of a new follicular wave (on average, 4.3 ± 0.1 d after treatment), regardless of the stage of development of the dominant follicle of the first follicular wave. Several studies, including those in this thesis, have evaluated the effect of longer acting forms of estrogen esters on follicular wave development and fertility in cattle (see Mapletoft et al., 2002a; 2003).

2.5 Control of estrous behaviour, gonadotrophin surge and ovulation

Some of the hormonal treatments used to induce new follicular wave emergence could be also used during pro-estrus to synchronize LH surge and ovulation. As described earlier, GnRH or LH treatment will result in ovulation if a dominant follicle ≥ 9 mm diameter is present at the time of treatment. Either hormone could be used to induce synchronous ovulation of a growing dominant follicle in addition to inducing emergence of the wave from which that follicle arose. GnRH is usually administered 48 h after PGF treatment in TAI protocols (Pursley et al., 1995; 1998). When GnRH was given during the follicular phase, ovulation was reported to occur between 24 and 32 h after treatment

(Pursley et al., 1998). When GnRH was administered during proestrus, ovulation of the dominant follicle was associated with a decrease in estradiol concentrations in the peripheral circulation (Twagiramungu et al., 1994) and the occurrence of spontaneous behavioural estrus was diminished (Martínez et al., 2002c). In addition to an absence of estrous behaviour, less uterine tone and cervical-vaginal fluid make cattle treated with GnRH more difficult to inseminate than those in spontaneous estrus.

Although LH has not been extensively used to synchronize ovulation, two studies investigated ovulatory response to different doses of pLH in heifers after PGF-induced luteolysis (Oswald et al., 2000; Ambrose et al., 2004). Ovulation of a dominant follicle >10 mm was similar whether heifers were given 5, 10, or 25 mg pLH 48 h after a PGF injection (overall, 96.4%; Oswald et al., 2000). Likewise, the interval from pLH treatment to ovulation did not differ among treatment groups (47 ± 10.5, 52.8 ± 13.4, and 38.4 ± 9.6 d for 5, 10, and 25 mg of pLH, respectively). Ambrose et al. (2004) reported a similar ovulatory response following administration of 5 or 12.5 mg pLH or 100 μg GnRH in CIDR-treated dairy heifers; however, the range of ovulation times was wider in heifers treated with 5 mg pLH.

The use of exogenous estradiol after termination of progesterone treatment was developed as a treatment regime to facilitate the expression of estrus and ovulation in cyclic (Wiltbank et al., 1971; Nancarrow and Radford, 1975) and anestrous (McDougall et al., 1992) cattle. Several authors have reported a decreased variation in time of onset of estrus (Nancarrow and Radford, 1975; Welch et al., 1975; Ryan et al., 1995; Hanlon et

al., 1996; 1997) and increased submission rates (Hanlon et al., 1996; Macmillan et al., 1996; Lamoglia et al., 1998) following estradiol treatment. However, pregnancy rates were reported to be variable with the use of estradiol following PGF treatment and/or progesterone withdrawal. In this regard, studies have shown a beneficial (Macmillan et al., 1996; Lammoglia et al., 1998), detrimental (Wiltbank et al., 1971) or no effect (Welch et al., 1975; Hanlon et al., 1996; Lane et al., 2001) on fertility. In more recent studies, estradiol treatment has been used to induce a synchronized LH surge and ovulation in cycling cattle allowing for TAI. The synchrony of estrus and ovulation after the administration of estradiol at various intervals after PGF treatment or progesterone withdrawal has been investigated. Administration of 1 mg of estradiol benzoate 24 h, but not 48 h after PGF treatment, reduced the interval to estrus (Ryan et al., 1995). A similar treatment given 10 (Ryan et al., 1999), 12 or 24 h (Martínez et al., 2002a) after CIDR removal resulted in more synchronous LH release and ovulation than that given at 36 h (Martínez et al., 2002a). Thus, the interval from estradiol treatment to the LH surge and ovulation may differ depending on when estradiol is given in relation to progesterone withdrawal (Martínez et al., 2002a), but not on stage of follicle development (Evans et al., 2003). Lammoglia et al. (1998) investigated different doses of estradiol benzoate in heifers and cows; optimal responses were at 0.38 mg for heifers and at 1 mg for cows. Regardless of time of treatment and dose, circulating estradiol concentrations peaked approximately 15 h after administration in ovariectomized (O'Rourke et al., 2000; Martínez et al., 2003a) or intact (Lammoglia et al., 1998; Martínez et al., 2002a) heifers, and LH peaked 16 to 20 h after estradiol administration (Bó et al., 1994; Lammoglia et al., 1998; Martínez et al., 2002a, 2003a).

Other studies investigated the effects of estradiol on the hypothalamo-pituitary axis. Estradiol appears to alter at least two parameters involved with inducing a preovulatory-like surge of gonadotrophins (Turzillo and Nett, 1999; Nett et al., 2002). Administration of estradiol increases the sensitivity of the pituitary gland to GnRH within 4 to 6 h (Kesner et al., 1981; Nett et al., 1984; Gregg et al., 1990; Turzillo et al., 1994), and then a sustained secretion of GnRH from the hypothalamus that is initiated 12 to 15 h after administration (Kesner et al., 1981; Moenter et al., 1991). Thus, the duration of the increased secretion of GnRH induced by estradiol supersedes the duration of LH surge (Moenter et al., 1991). Interestingly, the preovulatory LH surge induced by exogenous estradiol has been reported to last for approximately 10 h (Bó et al., 1994), which is similar to the spontaneous preovulatory LH surge (Chenault et al., 1975). However, the LH surge induced by an injection of GnRH had a duration of approximately 4 to 6 h (Chenault et al., 1990; Martínez et al., 2003b). Hence, CL function and subsequently pregnancy rate may be affected in those animals induced to ovulate by administration of GnRH compared to estradiol. Indeed, recent studies have shown a high incidence of short estrous cycles following GnRH-induced ovulations (Schmitt et al., 1996b; Cordoba and Fricke, 2002). In contrast, estradiol treatment seems to have no detrimental effect on CL function, even when small follicles are induced to ovulate by an injection of estradiol during proestrus (Evans et al., 2003). Although hormone treatments used to induce ovulation may affect CL morphology and capability to produce progesterone, and subsequent fertility, other studies have shown that reduced concentrations of estradiol near the time of ovulation may also affect oocyte maturation (Murdoch and Van Kirk,

2001) and oviductal and uterine environments (Boice et al., 1990; Murdoch and Van Kirk, 1998).

2.6 Effect on corpus luteum function

Treatment with GnRH during diestrus may affect function and morphology of the existing CL; an increase, decrease, or no change in progesterone production has been reported after treatment with GnRH or a GnRH agonist during diestrus (for review see Twagiramungu et al., 1995b). One study revealed that although plasma progesterone concentrations were not affected, numbers of large luteal cells (LLC) and the volume of the CL increased after buserelin (a potent GnRH agonist) treatment (Twagiramungu et al., 1995a). Conversely, treatment with LH or hCG not only increased the number of LLC (Farin et al., 1988), but it also increased plasma progesterone concentrations (Schmitt et al., 1996a; Diaz et al., 1998; Marques et al., 2002). Increase of plasma progesterone concentrations after hCG treatment was probably due to additional progesterone secretion by accessory CL (Schmitt et al., 1996a; Diaz et al., 1998). However, the effect on progesterone levels seems to be more prominent in hCG-treated animals than in those treated with GnRH or LH (Marques et al., 2002), likely due to stimulation of the original CL rather than to additional progesterone secretion by accessory CL (Fricke et al., 1993). It is likely that the increase in the number of LLC in GnRH- or hCG-treated animals is the result of an increased release of endogenous LH. Because PGF receptors are located on LLC (Fitz et al., 1982), the CL of animals treated with hCG, GnRH or pLH may become more sensitive to PGF treatment (Colazo et al., 2002b).

The luteolytic effects of exogenously administered estradiol esters in cattle was demonstrated several years ago (Wiltbank et al., 1961). The effect of estradiol on CL function was shown to be dose and estrous cycle stage dependent (Wiltbank et al., 1961; Munro and Moore, 1985) and mediated through uterine release of PGF (Peterson et al., 2000). The observation that the exogenous administration of estradiol caused follicle regression and wave emergence was quite coincidental and secondary to its intended use in the induction of luteolysis (Bó et al., 1991). Therefore, the use of estradiol to synchronize ovarian follicular wave emergence in cattle with unknown pregnancy status is of concern.

The current understanding of the endocrinology and physiology of reproduction in domestic animals has been briefly described. Also, methods to control ovarian follicle development and effects on CL function have been very briefly discussed. Based on this knowledge, studies in this thesis were initiated to develop and/or modify estrus synchronization protocols that allow for the use of artificial insemination without the necessity of estrus detection (TAI). It was anticipated that the development of TAI protocols that resulted in acceptable pregnancy rates would enhance the implementation of AI among beef producers. In addition, the development of synchronization protocols that permit reinsemination of non-pregnant animals with a minimum of estrus detection would noticeably increase the number of pregnant animals to AI in the first 24 d of the breeding season.

3.0 GENERAL HYPHOTHESIS

This thesis is based on the general hypothesis that controlling ovarian follicular growth and corpus luteum function in cattle would result in protocols for precise synchronization of estrus and ovulation and resynchronization (of nonpregnant cattle) with acceptable pregnancy rates.

4.0 GENERAL OBJECTIVE

The overall objective of the experiments reported herein was to develop alternative estrus synchronization and resynchronization protocols to facilitate the use of artificial insemination in cattle.

Specific objectives for each section of this thesis are indicated in the introduction preceeding each study.

5.0 EFFECTS OF ESTRADIOL CYPIONATE (ECP) ON OVARIAN FOLLICULAR DYNAMICS, SYNCHRONY OF OVULATION, AND FERTILITY IN CIDR-BASED, FIXED-TIME AI PROGRAMS IN BEEF HEIFERS

5.1 Abstract

Estradiol cypionate (ECP) was used in beef heifers receiving a CIDR (controlled internal drug release; insertion = Day 0) device for timed artificial insemination (TAI) in 4 experiments. In Experiment 1, heifers (n = 24) received 1 mg ECP or 1 mg ECP plus 50 mg of a commercial progesterone (CP) preparation im on Day 0. Eight or 9 d later, CIDR were removed, PGF was administered and heifers were allocated to receive 0.5 mg ECP im concurrently (ECP0) or 24 h later (ECP24). There was no effect of treatment (P = 0.6) on mean (± SEM) day of follicular wave emergence (3.9 ± 0.4 d). Interval from CIDR removal to ovulation was affected (P < 0.05) only by duration of CIDR treatment (88.3 ± 3.8 versus 76.4 ± 4.1 h; 8 versus 9 d, respectively). In Experiment 2, 58 heifers received 100 mg progesterone and either 5 mg estradiol-17β or 1 mg ECP im (E-17β and ECP groups, respectively) on Day 0. Seven (E-17β group) or 9 d (ECP group) later, CIDR were removed, PGF was administered and heifers received ECP (as in Experiment 1) or 1 mg estradiol benzoate (EB) 24 h after CIDR removal, with TAI 58 to 60 h after CIDR removal. Follicular wave emergence was later (P < 0.02) and more variable (P < 0.002)

in heifers given ECP than in those given E-17β (4.1 ± 0.4 versus 3.3 ± 0.1 d), but pregnancy rate was unaffected (overall, 69%; P = 0.2). In Experiment 3, 30 heifers received a CIDR device and 5 mg E-17β, with or without 100 mg progesterone im on Day 0. On Day 7, CIDR were removed and heifers received ECP as described in Experiment 1 or no estradiol (Control). Intervals from CIDR removal to ovulation were shorter (P < 0.05) in ECP0 (81.6 ± 5.0 h) and ECP24 (86.4 ± 3.5 h) groups than in the Control group (98.4 ± 5.6 h). In Experiment 4, heifers (n = 300) received a CIDR device, E-17β, progesterone, and PGF (as in Experiment 3) and after CIDR removal were allocated to 3 groups (as in Experiment 2), with TAI 54 to 56 h (ECP0) or 56 to 58 h (ECP24 and EB24) after CIDR removal. Pregnancy rate did not differ among groups (overall, 63.6%, P = 0.96). In summary, although 1 mg ECP (with or without progesterone) was less efficacious than 5 mg E-17β plus 100 mg progesterone for synchronizing follicular wave emergence, 0.5 mg ECP (at CIDR removal or 24 h later) induced a synchronous ovulation with an acceptable pregnancy rate following TAI.

5.2 Introduction

Estradiol-17β (E-17β) and estradiol benzoate (EB) are commonly used to synchronize follicular wave emergence (Bó et al., 1994; Caccia and Bó, 1998; Martínez et al., 2000a, 2000b) and ovulation (Hanlon et al., 1996, 1997; Colazo et al., 1999; Martínez et al., 2002a) in CIDR-treated cattle. However, estradiol cypionate (ECP), an ester of estradiol with a low water solubility that delays its release from the site of injection, is the only estrogen ester licensed for use in cattle in North America. Plasma

concentrations of estradiol were prolonged (98 to 170 h) after intramuscular administration of large doses (5 or 10 mg) of ECP (Burton et al., 1990; Vynchier et al., 1990). However, when ovariectomized beef heifers were treated with only 2 mg ECP, the duration of estrus (15.8 ± 1.1 h) was not different from heifers with a spontaneous estrus (Lefebvre and Block, 1992), suggesting that reduced doses of ECP may be useful in estrus synchronization regimens.

The effects of ECP on ovarian follicular wave emergence and ovulation have been studied in lactating Holstein cows (Thundathil et al., 1997). Although 1 mg ECP was more efficacious than 0.5 mg in synchronizing follicular wave emergence, the authors concluded that the prolonged half-life of ECP made it far less efficacious than E-17β for synchronizing both follicular wave emergence and ovulation in CIDR-treated dairy cattle. However, Lopes et al. (2000a) successfully used ECP to replace the second treatment with GnRH to synchronize ovulation for fixed-time AI in an Ovsynch-type program in dairy cattle. Furthermore, Ambrose et al. (2001) reported that 0.5 mg of ECP administered concurrently with CIDR removal synchronized ovulation in Holstein heifers that had received PGF 24 h before CIDR removal. To our knowledge, the use of ECP in comparison or combination with E-17β or EB for timed AI (TAI) in beef cattle has not been reported.

Four experiments were designed to study and compare the effects of ECP (with or without injectable progesterone), E-17β, and EB on follicular dynamics, time of ovulation, and pregnancy rate following TAI in CIDR-based protocols. In addition, the

duration of CIDR treatment (8 versus 9 d) on the interval from CIDR removal to ovulation was determined.

5.3 Materials and Methods

5.3.1 Experiment 1

Puberal, crossbred beef heifers 18 to 20 mo of age (n = 24), received a once-used intravaginal progesterone releasing device (CIDR; Bioniche Animal Health; Belleville, ON, Canada) at random stages of the estrous cycle, and were randomly assigned to 2 groups to concurrently receive either 1 mg of estradiol cypionate im (ECP; Pharmacia Animal Health, Orangeville, ON, Canada; n = 13) or 1 mg ECP plus 50 mg of a commercial progesterone preparation (CP; Progesterone 5%®, J Webster Lab Inc, Victoriaville, QC, Canada; n = 11) on Day 0 (day of treatment). Heifers were randomly allocated to have the CIDR removed and receive treatment with 500 μg cloprostenol im (Estrumate, Schering Plough Animal Health, Pointe-Claire, QC, Canada) on either Day 8 (n = 13) or Day 9 (n = 11). Heifers were further randomly allocated to receive 0.5 mg of ECP at CIDR removal (ECP0; n = 13) or 24 h later (ECP24; n = 11).

Ovaries were examined by transrectal ultrasonography (Aloka SSD 500 with a 7.5 MHz linear-array transducer; ISM Inc., Edmonton, AB, Canada) once daily from CIDR insertion (Day 0) to 48 h after CIDR removal to detect ovarian dynamics, and thereafter, twice daily to determine the time of ovulation. The diameter of the CL and all follicles ≥3

mm were measured and recorded (Pierson and Ginther, 1984). The day of emergence of a follicular wave was defined as the day that the dominant follicle was first identified at a diameter of 4 mm (Ginther et al., 1989b). When follicular wave emergence did not occur during the observation period, day of follicular wave emergence prior to treatment was estimated from the size of the dominant follicle at the time of treatment. Ovulation was confirmed by the disappearance of a large (>10 mm) follicle that had been detected at the previous examination.

5.3.2 Experiment 2

Fifty-eight puberal, crossbred heifers were treated with a once-used CIDR device and 100 mg progesterone in canola oil im (Sigma Chemical Co, St Louis, MO, USA) and randomly assigned to 2 groups to receive either 5 mg of E-17β im (E17β; Sigma Chemical Co; n = 30) or 1 mg of ECP im (ECP; n = 28) to synchronize follicle wave emergence (Day 0). The CIDR devices were removed after 7 or 9 d in the E17β and ECP groups, respectively and 25 mg dinoprost im (Lutalyse; Pharmacia Animal Health) was administered. Heifers were further divided into 3 groups in a 2 x 3 factorial design to receive ECP as in Experiment 1 (ECP0; n = 19 or ECP24; n = 20), or 1 mg of estradiol benzoate (EB; Sigma Chemical Co; EB24, n = 19) 24 h after CIDR removal. All heifers were inseminated using TAI 58 to 60 h after CIDR removal. Ultrasonographic examinations were performed to determine ovarian dynamics and ovulation as described in Experiment 1 and 28 d after AI for pregnancy diagnosis.

5.3.3 Experiment 3

Puberal, crossbred beef heifers, 18 to 20 mo of age (n = 30), received a CIDR device (insertion = Day 0) at random stages of the estrous cycle. Concurrently, heifers were given 5 mg of E-17β im, alone or in addition to 100 mg of progesterone in canola oil im to synchronize follicle wave emergence. On Day 7, CIDR devices were removed; heifers were treated with 25 mg dinoprost im and randomly allocated into 3 groups to receive no treatment (Control; n = 10) or 0.5 mg ECP im (ECP0; n = 10 or ECP24; n = 10) as in Experiment 1.

Ultrasonographic examinations of the ovaries were conducted as described in Experiment 1. Heifers were observed for estrous behavior every 6 h, from 24 h after CIDR removal to ovulation. Blood samples were collected by jugular venipuncture into an evacuated tube containing heparin every 6 h from CIDR removal to detection of ovulation. Blood samples were centrifuged for 20 min at 1500 x g, and plasma was stored at –20° C until assayed. Plasma LH concentrations were determined by radioimmunoassay (Rawlings et al., 1984). The intra-assay coefficients of variation were 7.2 and 6.5% for means of 0.38 and 1.04 ng/mL respectively.

5.3.4 Experiment 4

Angus crossbred beef heifers (n = 300), approximately 13 to 15 mo of age and 350 to 500 kg, were kept in outdoor paddocks under feedlot conditions. Based on

ultrasonographic examination of the ovaries at the beginning of the experiment, heifers that did not have a luteal structure were deemed prepuberal and equally allocated among treatment groups. All heifers received a CIDR, E-17β plus progesterone and PGF (as in Experiment 3) and were allocated to receive ECP (ECP0; n = 98 or ECP24; n = 99) or EB (EB24; n = 103) as described in Experiment 2. Heifers in the ECP0 group were inseminated using TAI between 54 and 56 h after CIDR removal, while heifers in the other 2 groups (ECP24 and EB24) were inseminated between 56 and 58 h after CIDR removal. Pregnancy diagnosis was done as described in Experiment 2.

5.3.5 Statistical Analyses

For Experiments 1 to 4, all statistical analyses were done with commercial statistical software (SPSS for Windows, Version 10.05; Analytical Sofware, 2001, Chicago, IL, USA). Single-point variables were analyzed by ANOVA and differences among groups were identified with a protected LSD test. Student's t-test was used to compare day of follicular wave emergence between treatments (Rank Sum Test was used in Experiment 2). Comparison of equality of variances among groups was done by Bartlett's test of homogeneity of variance. Continuous variables were compared by group with ANOVA for repeated measures to determine the main effects of treatment and day, and their interaction. Pearson correlation coefficients were calculated between diameter of the eventual ovulatory follicle (on Days 8, 9, and just prior to ovulation), timing of follicular wave emergence, and intervals to estrus, LH surge, and ovulation. An LH surge

was defined as an increase in plasma LH concentration ≥ 2 SD above the overall LH mean. In Experiment 3, proportional data were compared by Chi-square analysis.

5.4 Results

5.4.1 Experiment 1

Two heifers, one in each treatment group, did not respond to treatment to induce follicular wave emergence at CIDR insertion and they were excluded from the calculation of the interval from treatment to wave emergence. There was an effect of day ($P < 0.0001$) on diameter of the regressing follicle, but the effects of treatment ($P = 0.17$), and the treatment by day interaction ($P = 0.66$) were not significant. Similarly, the effects of day ($P = 0.35$), treatment ($P = 0.99$) and the treatment by day interaction ($P = 0.9$) on CL diameter were not significant. There was no difference ($P = 0.6$) between treatment groups for the day of emergence of a new follicular wave (3.7 ± 0.4 and 4.0 ± 0.5 d for ECP and ECP/CP groups, respectively) and so groups were combined. Overall, a new follicular wave emerged between 2 and 7 d after treatment.

The mean (± SEM) diameter of dominant follicle at the time of CIDR removal was numerically smaller in heifers treated with a CIDR for 8 days (10.8 ± 2.8 mm) than in heifers treated for 9 days (12.8 ± 2.8 mm), although this difference was not statistically significant ($P = 0.11$). Similarly, the diameter of dominant follicle prior to ovulation was

not different between groups (P = 0.46; 15.3 ± 2.0 and 16.0 ± 2.3 mm for heifers in 8 and 9 d groups, respectively).

The interval from CIDR removal to ovulation was shorter (P < 0.05) in heifers treated with a CIDR for 9 d (76.4 ± 4.1 h) than those treated for 8 d (88.3 ± 3.8 h). Two heifers treated with a CIDR for 9 d ovulated by 60 h after CIDR removal. Heifers administered ECP at CIDR removal had a numerically shorter interval (although not significant; P = 0.16) from CIDR removal to ovulation (78.5 ± 3.5 h) than those treated with ECP 24 h later (87.3 ± 5.1 h). In heifers receiving a CIDR for 8 d, ECP treatment at CIDR removal tended to shorten (P < 0.09) the interval between CIDR removal and ovulation (80.6 ± 5.0 h) compared to ECP treatment 24 h later (96.0 ± 7.0 h). However, mean (± SEM) intervals from CIDR removal to ovulation were similar (P = 0.9; 76.0 ± 5.0 and 76.8 ± 4.8 h for ECP0 and ECP24 groups, respectively) in heifers treated with a CIDR for 9 d.

5.4.2. Experiment 2

One heifer treated with ECP did not respond with emergence of a new follicular wave and was not included in the calculation of the interval from treatment to emergence of a new follicular wave. There was an effect of day (P < 0.0001), but the effects of treatment (P = 0.58; P = 0.28), and treatment by day interaction (P = 0.45; P = 0.56) were not significant for diameter of growing and regressing follicles, respectively (Figure 5.1).

However, there was an effect of day ($P < 0.0001$) and treatment ($P < 0.02$) on CL diameter (Figure 5.1).

Mean (± SEM) day of follicular wave emergence was delayed ($P < 0.02$) and more variable ($P < 0.002$) in heifers given ECP compared to those given E-17β (4.1 ± 0.4 d versus 3.3 ± 0.1 d). The proportion of heifers with emergence of a new follicular wave on Days 3 or 4 after treatment tended to be higher ($P < 0.06$) in heifers treated with E17β (24/30; 80%) than in heifers treated with ECP (16/28; 57%). Dominant follicle diameter at the time of CIDR removal was larger ($P < 0.01$) in ECP-treated heifers than E-17β-treated heifers (11.3 ± 2.1 versus 9.8 ± 1.5 mm). Variances were also different ($P < 0.04$) between groups (ranges, 6.5 to 14.2 mm and 7.0 to 13.2 mm for ECP and E-17β groups, respectively). However, there was no difference in pregnancy rates among groups (overall, 69%; $P = 0.2$; Table 5.1). Although there was no difference ($P = 0.3$) among treatments to synchronize ovulation, the ECP24 group had a numerically higher pregnancy rate (16/20) than the other two groups (ECP0, 11/19 and EB24, 13/19). Pregnancy rates did not differ ($P = 0.86$) between heifers treated with 100 mg of progesterone plus either 5 mg of E-17β (21/30) or 1 mg of ECP (19/28) to synchronize follicular wave emergence.

Five heifers (9% of the total) ovulated prior to fixed-time AI (58 to 60 h after CIDR removal; 3 heifers from the ECP0 group, 1 from the EB24 group, and 1 from the ECP24 group). The heifers in the ECP0 and EB24 groups were subsequently diagnosed not pregnant, while the heifer in ECP24 group was pregnant. In contrast, 38 of 49 heifers

(78%) that ovulated ≤ 24 h after AI were pregnant, regardless of treatment. Of the 4 heifers (7% of the total) that presumably ovulated after 24 h, 2 became pregnant. It is noteworthy that 13% (5/39) of the heifers receiving an ECP treatment either at CIDR removal (n = 2) or 24 h later (n = 3) had double ovulations.

Table 5.1. Pregnancy rates in heifers after treatment with 100 mg of progesterone plus 5 mg of estradiol-17β (E-17β/) or 1 mg of estradiol cypionate (ECP) to synchronize follicular wave emergence, and 0.5 mg ECP at CIDR removal or 24 h later (ECP0 and ECP24, respectively) or 1 mg estradiol benzoate 24 h after CIDR removal (EB24) to synchronize ovulation for timed AI.

	Heifers	
Treatment	inseminated (n)	pregnant (n)
E-17β/ECP0	10	7
E-17β/ECP24	10	6
E-17β/EB24	10	8
ECP/ECP0	9	4
ECP/ECP24	10	10
ECP/EB24	9	5

There was no difference in pregnancy rates among treatments ($P < 0.2$).

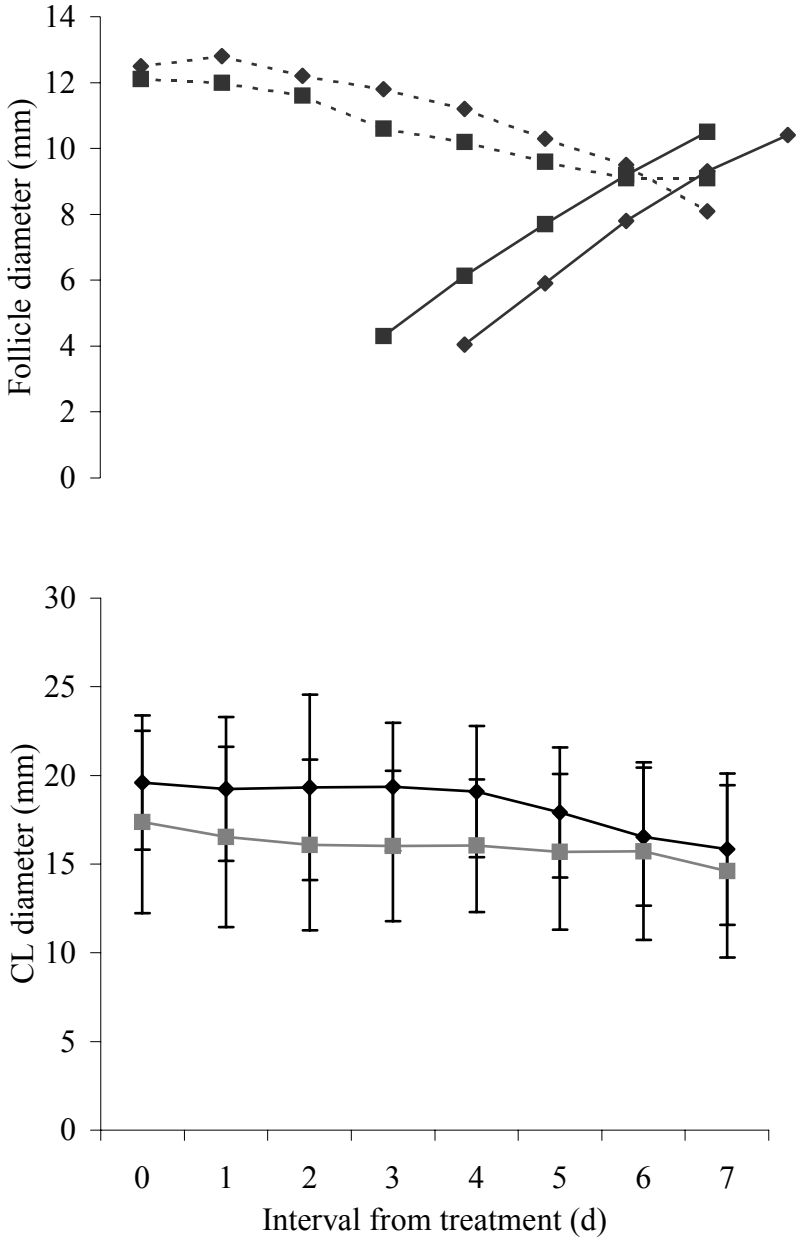

Figure 5.1. Mean diameters (upper panel) for the dominant follicle that regressed (---) and the eventual ovulatory follicle (—) after treatment with either 1 mg estradiol cypionate plus 100 mg progesterone (♦;ECP/P, n = 27) or 5 mg estradiol-17β plus 100 mg progesterone (■; E-17β/P, n = 30) at CIDR insertion (Day 0). Day of follicular wave emergence was normalized to average day of emergence. Mean (± SEM) CL diameter for heifers in the ECP/P (♦) and E-17β/P (■) groups is shown in the lower panel. There was no effect of treatment on the diameter of regressing (P = 0.58) or growing (P = 0.28) follicles. However, there were effects of day (P < 0.0001) and treatment (P < 0.02) on CL diameter.

5.4.3 Experiment 3

Three heifers that did not respond to the first treatment (to induce a new follicular wave) were excluded from the statistical analyses. Means ($P = 0.4$) and variances ($P = 0.11$) for day of emergence of a new follicular wave were not different between treatment groups (overall, 3.4 ± 0.7 d). There was an effect of day ($P < 0.0001$) on diameter of the dominant follicle at the time of CIDR removal, but the effects of treatment ($P = 0.28$), and treatment by day interaction ($P = 0.85$) were not significant.

Overall, heifers receiving ECP had a shorter ($P < 0.05$) interval from CIDR removal to estrus and ovulation than control heifers (49.3 ± 3.4 and 81.6 ± 5.0 h; 51.6 ± 2.8 and 86.4 ± 3.5 h; 60.0 ± 3.7 and 98.4 ± 5.6 h in ECP0, ECP24 and control groups, respectively). Nineteen of 20 heifers in ECP groups ovulated between 72 and 96 h after CIDR removal (1 heifer in the ECP0 group ovulated at 48 h).

Examination of LH concentrations on an individual-animal basis in heifers given ECP (both groups) revealed that LH surges occurred at either 42 to 48 or 60 to 66 h after CIDR removal (data not shown). The interval from CIDR removal to the LH surge tended ($P < 0.07$) to be shorter in heifers receiving ECP than for those in the control group (Table 5.2). The mean ($\pm$ SEM) interval from the LH surge to ovulation did not differ among groups (30.0 ± 1.4, 28.6 ± 1.2, and 28.7 ± 1.7 h for control, ECP0, and ECP24 groups, respectively; $P < 0.7$), but LH surge concentrations were lower ($P < 0.01$)

in heifers in the control group than in those in the ECP groups (6.8 ± 0.6, 16.2 ± 2.3, and 17.2 ± 4.1 ng/mL for control, ECP0, and ECP24 groups, respectively.

Day of emergence of a new follicular wave was negatively correlated with diameter of the eventual ovulatory follicle on Days 8 and 9 after treatment (r = - 0.63 and r = - 0.62; P < 0.0008), and positively correlated with the timing of the LH surge (r = 0.51; P < 0.007). However, the interval to the LH surge was negatively correlated with the diameter of the eventual ovulatory follicle on Day 8 (r = - 0.39; P < 0.05) and positively correlated with the interval to ovulation (r = 0.94; P < 0.001). In addition, the interval to ovulation tended to be correlated with day of emergence of a new follicular wave (r = 0.37; P < 0.06) and diameter of the eventual ovulatory follicle on Day 8 (r = - 0.34; P < 0.09).

Table 5.2. Mean (± SEM) intervals from CIDR removal to estrus, LH surge, and ovulation in beef heifers given 0.5 mg estradiol cypionate (ECP) at 0 or 24 h after CIDR removal (ECP0 and ECP24, respectively) or no treatment (Control).

	ECP0	ECP24	Control
Interval (h) from CIDR removal to			
Estrus	49.3 ± 3.4[a]	51.6 ± 2.8[a]	60.0 ± 3.7[b]
(Range)	(36 to 66)	(36 to 60)	(36 to 72)
LH surge	54.6 ± 3.4	59.3 ± 3.5	68.0 ± 4.7
(Range)	(42 to 66)	(42 to 72)	(54 to 96)
Ovulation	81.6 ± 5.0[a]	86.4 ± 3.5[a]	98.4 ± 5.6[b]
(Range)	(48 to 96)	(72 to 96)	(72 to 132)

[ab] Within a row, means with different superscripts are different (P < 0.05).

5.4.4 Experiment 4

Forty-nine heifers (16.3%) were prepuberal at the beginning of the experiment, but pregnancy rate (61.2%) was not different from those that were puberal (64.1%; P = 0.7). Hence, pregnancy data from all heifers were combined. Pregnancy rates did not differ among groups (62/98, 63.3%, 64/99, 64.6%, and 65/103, 63.1% for ECP0, ECP24, EB24 groups, respectively; P = 0.96).

5.5 Discussion

Although the administration of 1 mg ECP resulted in relatively synchronous follicular wave emergence in the present study, synchrony was less precise than with 5 mg of E-17β; this is consistent with a previous study in which the administration of ECP to dairy cows resulted in relatively asynchronous follicular wave emergence (Thundathil et al., 1997). Similarly, Bó et al. (1996) reported delayed or asynchronous emergence of a new follicular wave when 5 mg of EB (another estradiol ester) was administered. However, treatment with 1 or 2.5 mg EB resulted in synchrony of follicular wave emergence that did not differ from that of 5 mg E-17β (Caccia and Bó, 1998; Martínez et al., 2002a).

Plasma FSH concentrations are suppressed by negative feedback from estradiol and inhibin released from actively growing follicles. Furthermore, periodic surges in circulating FSH concentration consistently precede follicular wave emergence (Adams et

al., 1992a; Bergfelt et al., 1994a). In ECP-treated heifers, elevated plasma estradiol concentrations may have suppressed endogenous FSH release for longer and more variable intervals compared to that in heifers given E-17β, leading to delayed follicular wave emergence. Prolonged elevations in plasma concentrations of estradiol following treatment with ECP have been previously reported (Burton et al., 1990; Vynchier et al., 1990), while plasma estradiol concentrations following treatment with E-17β increased and declined much more rapidly (Bó et al., 2000; Ginther et al., 2000).

Despite the relatively asynchronous wave emergence observed in heifers treated with ECP, ovulation was relatively synchronous and pregnancy rate following TAI did not differ (21/30 and 19/28 for E17β and ECP, respectively). In a previous study, Kastelic and Mapletoft (1998) reported that although treatment with GnRH resulted in a variable interval to follicular wave emergence, cloprostenol given 6 d after GnRH resulted in a relatively synchronous ovulation, even in the absence of a second treatment with GnRH. It is important to note that all heifers in the present study were given estradiol (ECP or EB) to synchronize ovulation. We have previously shown the importance of giving estradiol after CIDR removal to achieve high pregnancy rates following TAI (Colazo et al., 1999).

Estradiol cypionate treatment at the time of CIDR removal or 24 h later effectively synchronized the LH surge and ovulation; consistent with this, pregnancy rates did not differ among treatments (ECP0, ECP24 or EB24) in Experiment 4. Nevertheless, in Experiment 2, ECP treatment 24 h after CIDR removal resulted in a

numerically higher pregnancy rate than ECP treatment at device removal (where several precocious ovulations occurred in the latter group). Overall, heifers that ovulated early (i.e. before AI or 60 h after CIDR removal) either had a regressed or regressing CL and a large dominant follicle (≥ 11.2 mm) at the time of CIDR removal. As progesterone concentrations were undoubtedly low in these heifers (Kastelic et al., 1990b), estradiol treatment or an estrogen-active dominant follicle may have induced an early LH surge, which induced ovulation before the expected time.

Recently, Ambrose et al. (2001) reported high pregnancy rates following TAI 48 h after CIDR removal in Holstein heifers administered ECP concurrently with CIDR insertion and withdrawal and administration of PGF 24 h before CIDR removal, whereas the ECP/ECP0 group in the present study had numerically, the lowest percentage of pregnant heifers; early ovulations may have adversely affected pregnancy rate in this group. However, administration of PGF 24 h before CIDR removal may have also resulted in more synchronous ovulations, resulting in higher pregnancy rates (Ambrose et al., 2001). In the present study, the administration of ECP at CIDR removal (to synchronize ovulation) was intended to eliminate handling required to administer estradiol 24 h after CIDR removal. Interestingly, administration of 0.5 mg ECP at the time of CIDR removal in heifers treated with E-17β at CIDR insertion (Experiments 2 and 4) did not adversely affect pregnancy rate; this approach must be further tested before widespread implementation.

When CIDR devices were removed after 8 or 9 d, the latter group had a shorter interval from CIDR removal to ovulation, and a larger (although not significant) dominant follicle diameter at the time of CIDR removal. One additional day of treatment represented, on average, 2 mm more in follicular growth. In that regard, the diameter of the eventual ovulatory follicle at the time of PGF treatment has been shown to be negatively correlated with the interval from PGF treatment to estrus and to ovulation (Kastelic et al., 1991; Colazo et al., 2002b). Hence, the length of the treatment in CIDR-based estrus synchronization programs should be taken in account when scheduling TAI.

In these experiments, all heifers that failed to respond to the first estradiol/progesterone treatment developed a persistent follicle (Savio et al., 1993). Ovulation of persistent follicles has been shown to result in reduced conception rates after AI (Ahmad et al., 1995; Kinder et al., 1996; Revah and Butler, 1996); therefore, it is important to minimize the occurrence of persistent follicles in estrus synchronization protocols. It is noteworthy that non-responding heifers in the present study were either at late stages of the luteal phase or proestrus when treatment was initiated. In this regard, Martínez et al. (2002a) have also reported that the dominant follicle of 2 beef heifers undergoing spontaneous luteal regression at the time of CIDR insertion and treatment with either 5 mg E-17β or 1 mg EB plus 100 mg progesterone did not regress. Collectively, results suggest that in heifers undergoing spontaneous luteolysis at the time of CIDR insertion, estradiol/progesterone treatment will not consistently induce regression of the dominant follicle, and ovulation of an aged oocyte following CIDR removal may result in reduced pregnancy rates following TAI. Therefore, the stage of the

estrous cycle at initiation of estradiol treatment may be an important factor affecting the synchronization of follicular wave emergence, and following CIDR removal, synchronous ovulation of a viable oocyte.

In summary, ECP was less efficacious than E-17β for synchronization of follicular wave emergence in CIDR-treated heifers. However, pregnancy rate to fixed-time AI was comparable to that achieved in these and previous studies using the shorter-acting estradiol formulations. In addition, results suggest that the administration of 0.5 mg ECP at CIDR removal will induce synchronous ovulation of the dominant follicle of a follicular wave synchronized with E-17β. Nevertheless, ECP treatment 24 h after CIDR removal was a highly efficacious alternative for heifers that received ECP at the time of CIDR insertion to synchronize follicular wave emergence. These important observations must be investigated further to reduce animal handling without compromising pregnancy rates following TAI.

6.0 FERTILITY FOLLOWING TIMED AI IN CIDR-TREATED BEEF HEIFERS GIVEN GNRH OR ESTRADIOL CYPIONATE AND FED DIETS SUPPLEMENTED WITH FLAX SEED OR SUNFLOWER SEED

6.1 Abstract

The objectives were to determine pregnancy rates following timed AI (TAI) in heifers: 1) given GnRH or estradiol cypionate (ECP) to synchronize follicular wave emergence and ovulation in a CIDR-based protocol; and 2) fed diets supplemented with flax or sunflower seeds. At two locations, Angus and crossbred Angus heifers (n=983) were examined ultrasonically to confirm reproductive maturity and randomly allocated to six synchronization groups in a 2 x 3 factorial design. On Day 0 (start of synchronization treatments), heifers received a CIDR and either 100 μg GnRH im (n = 492) or 1 mg ECP plus 50 mg progesterone im (n = 491); in these groups, CIDR removal and PGF treatment were done concurrently on Days 7 and 8.5, respectively. Heifers were re-randomized to receive 0.5 mg ECP im at CIDR removal or 24 h later (with TAI 58 to 60 h after CIDR removal in both groups), or 100 μg GnRH im concurrent with TAI (52 to 54 h after CIDR removal). The heifers were fed a barley silage-based diet for 50 d (from Day –25 to 25) supplemented with 1 kg/heifer/d of flax seed (n = 321), sunflower seed (n = 324), or no oilseed (n = 338). Pregnancy rate following TAI (overall, 56.2%) was not affected by treatment at CIDR insertion (P=0.96) but was higher (P<0.005) in heifers given ECP

24 h after CIDR removal (216/330, 65.4%) than in those given either ECP at CIDR removal (168/322, 52.1%) or GnRH at AI (169/331, 51.1%). Overall, there was no effect of diet on pregnancy rates (P=0.46). In summary, pregnancy rate following TAI was not significantly affected by treatment at CIDR insertion to synchronize follicular wave emergence, but 0.5 mg ECP 24 h after CIDR removal (to synchronize ovulation) resulted in the highest pregnancy rate.

6.2 Introduction

In progestin-based protocols for timed AI (TAI), GnRH, estradiol benzoate (EB), and estradiol-17β (E-17β) have all been used to synchronize follicular wave emergence and ovulation (Adams et al., 1995; Colazo et al., 1999; Martínez et al., 2000a; 2002b). Although GnRH is licensed for use in cattle in North America, neither EB nor E-17β are licensed. In contrast, estradiol cypionate (ECP) is a licensed product. In an initial study, follicular wave emergence following administration of ECP in CIDR-treated dairy cows was quite asynchronous (Thundathil et al., 1997). However, ECP has recently been successfully used to synchronize both wave emergence and ovulation in CIDR-treated dairy (Ambrose et al., 2001) and beef (Colazo et al., 2003b, Chapter 5) heifers. In these studies, ECP was given either at CIDR removal (Ambrose et al., 2001) or 24 h later (Colazo et al., 2003b, Chapter 5) to synchronize ovulation.

The addition of specific long-chain, polyunsaturated fatty acids to cattle diets, particularly those of the omega-3 family, may increase fertility (Staples et al., 1998; Mattos et al., 2000). Furthermore, dietary supplementation with ruminant-grade menhaden fish-meal improved conception rates in lactating dairy cows (Burke et al., 1997a). Menhaden fish-meal is a rich source of eicosapentaenoic and docosahexaenoic fatty acids, known to reduce PGF release from the uterus (Thatcher et al., 1997; 2001). Feeding flax seed to dairy cows has been reported to increase the proportion of linolenic acid in milk fat (Kenelly, 1996). Flax seed is a rich source of α-linolenic acid (Ambrose and Kastelic, 2003). Supplementation with α-linolenic acid can lead to the formation of eicosapentaenoic acid and therefore has the potential to reduce PGF secretion and early embryo mortality. In two recent studies, the inclusion of flax seed in the diet of lactating dairy cows was associated with increased conception rates (Petit et al., 2001). Unlike flax seed, the α-linolenic acid content in sunflower seed is very low, but it is rich in linoleic acid (Ambrose and Kastelic, 2003). While linoleic acid intake can increase the supply of arachidonic acid, a precursor to PGF synthesis, it can also have inhibitory effects on PGF synthesis and possibly enhance reproductive performance in cattle (Staples et al., 1998).

The objectives of the present study were to determine pregnancy rates following TAI in heifers: 1) given GnRH or ECP (to synchronize follicular wave emergence and ovulation) in a CIDR-based protocol; and 2) fed diets supplemented with flax or sunflower seeds.

6.3 Materials and Methods

The study was replicated simultaneously at two feedlots, approximately 50 km apart. Angus and Angus cross heifers 12 to 15 mo of age and weighing approximately 325 to 375 kg were used. Heifers were allocated to the following dietary groups: Flax seed, Sunflower seed and control. Oilseed supplementation commenced approximately 25 d before initiation of the estrus synchronization program and continued for 50 d. Heifers in both locations were fed a base ration of barley silage and approximately 10% of either barley grain (Location A) or malt-sprout pellets (Location B). In heifers assigned to oil seed diets, 1.0 kg of either whole flax seed or sunflower seed, respectively, replaced 1.5 kg of barley or malt sprout pellets. Diets were delivered once daily in the form of a total mixed ration. Heifers were limit-fed and consumed an estimated 7.2 to 9.0 kg of feed per head per day on a dry matter basis. Heifers had ad libitum access to clean drinking water, cobalt iodized salt, and a calcium/phosphorous mineral supplement.

Just prior to the start of the synchronization protocols, heifers were examined by transrectal ultrasonography (Aloka SSD 500 with 7.5 MHz linear-array transducer; ISM Inc., Edmonton, AB, Canada). Freemartin heifers and those without an ultrasonically detectable luteal structure and (or) ovarian follicles <10 mm in diameter and a uterine diameter <15 mm were excluded. A total of 983 heifers were assigned to the experiment (flax seed, n = 321; sunflower seed, n = 324; and control, n = 338). All heifers received a new intravaginal progesterone-releasing device (CIDR; Bioniche Animal Health Inc.,

Belleville, ON, Canada) and were randomly allocated to one of six synchronization treatments in a 2 x 3 factorial design. At CIDR insertion (designated Day 0), heifers received either 100 μg GnRH im (Fertagyl; Intervet Canada Ltd., Whitby, ON, Canada; n = 492) or 1 mg estradiol cypionate (ECP; Pharmacia Animal Health, Orangeville, ON, Canada) plus 50 mg Progesterone 5%® im (Vétoquinol N.-A Inc., Lavaltrie, QC, Canada) to synchronize follicle wave emergence (n = 491). On Day 7 and 8.5 in heifers given GnRH or ECP, respectively, the CIDR devices were removed and 25 mg dinoprost (Lutalyse; Pharmacia Animal Health) was given im. Heifers were then randomly allocated to three groups to receive: 0.5 mg ECP im at CIDR withdrawal (ECP0, n = 322) or 24 h later (ECP24, n = 330) or 100 μg GnRH im (GnRH52, n = 331) at TAI (approximately 52 to 54 h after CIDR removal). In the ECP0 and ECP24 groups, TAI was done between 58 and 60 h after CIDR removal. All inseminations at each location were performed by one technician, using commercial frozen-thawed semen. To facilitate treatments and handling, TAI was done on three consecutive days, with heifers in the ECP24, ECP0 and GnRH52 bred on the first, second and third days, respectively. Care was taken to handle each group in a similar fashion to minimize a day of breeding effect not attributable to treatment differences.

Following TAI, heifers were observed for estrus and reinseminated until 33±1 d after TAI, at which time heifers were sent to pasture and exposed to bulls for 57 d; all bulls used were deemed satisfactory on a standard breeding soundness evaluation (Barth, 2000). Mounting activity was noted approximately 4 d after TAI at Location B; overall, 19 heifers were detected in estrus and re-inseminated at that time. All were considered

open following TAI. It is noteworthy that 17/19 (89.4%) of these heifers were in the GnRH52 group.

Ultrasonographic pregnancy diagnosis was conducted approximately 28 d after TAI. Fall pregnancy rate was determined approximately 150 d after TAI by rectal palpation. Fall pregnancy rate included all pregnancies (achieved by AI or natural service).

Oilseeds, bulk-sampled once during the course of the trial, were analyzed for moisture, crude protein, crude fat and crude fibre in a commercial laboratory. Fatty acid methyl esters (FAME) in the oil seeds were prepared by a modification of previously reported procedures (Sukhija and Palmquist, 1990; Chin et al., 1992). To a 0.5 g sample, 1 mL of hexane, 1 mL of nonadecanoic acid internal standard (C17:0, 4 mg/mL in hexane), and 3 mL of fresh 4% methanolic HCl were added. Tubes were tightly capped, vortexed, and heated for 1 h at 60 ^{o}C. After cooling, 5 mL of 6% K2CO3 and 2 mL of hexane were added to the tubes, which were vortexed and centrifuged. To the hexane extract, 1 g of Na2SO4 and 1 g of activated charcoal were added, vortexed and centrifuged again, and an aliquot of the clear hexane layer was used for gas chromatography. The FAME were analyzed on a Varian 3600 gas chromatograph (Varian Chromatography Systems, Walnut Creek, CA, USA) with a septum programmable injector and flame ionization detector. Separation of the FAME was performed using an AP2560 (100m x 0.25 id x 0.2 μm film thickness) fused silica capillary column (Supelco, Bellefonte, PA, USA). Purified helium (Praxair, Edmonton, AB, Canada) was used as the

carrier gas with a head pressure of 25 psi and a flow rate of 1 mL/min. The initial column temperature was set at 40 ^{0}C and held for 4 min, increased to 175 ^{0}C at 13 ^{0}C per min and held for 25 min, further increased to 215 ^{0}C at 4 ^{0}C per min and held for 23 min, and finally increased to 230 ^{0}C at 5 ^{0}C per min and held for 17.5 min. The initial injector temperature was 100 ^{0}C, increasing at 150 ^{0}C per min to 250 ^{0}C and held for 30 min. The detector temperature was set at 250 ^{0}C. Identification of peaks was based on comparison with retention times of standard FAME (GLC #85 and GLC #411, NuChek Prep, Elysian, MN, USA). Peak areas of each fatty acid were measured using the Scimatzu Class-VP chromatography data system (Shimatzu Scientific Instruments Inc, Columbia, MD, USA) and reported as a percentage of fatty acid methyl esters.

6.3.1 Statistical Analyses

The association between pregnancy status and treatment group, location, and diet were analysed using a General Estimating Equations (GEE) method to account for the clustering due to the pen effect. Data were analyzed with a statistical software program (PROC GENMOD; SAS Version 8.2 for Windows, SAS Institute, Cary, NC, USA). Model specifications included a binomial distribution, logit link function, repeated statement with subject equal to pen, and an exchangeable correlation structure. The treatment given at CIDR insertion (GnRH versus ECP) was analyzed as a fixed effect with two categories, and the treatment given to synchronize ovulation (ECP0, ECP24 and GnRH52) was analyzed as a fixed effect with three categories. Location and diet were considered as possible covariates and analyzed as categorical fixed effects. Variables

remaining in the final multivariable model at $P < 0.05$, based on the robust empirical standard errors produced by the GEE analysis, were considered statistically significant. The main-effects model was assessed for first-order interactions, where treatment and location or diet remained in the model. Only interactions with $P < 0.05$ were reported. Model diagnostics included visual examination of the raw and standardized residuals. The residuals were plotted against predicted values of each observation. Rankit plots and Wilk-Shapiro tests were used to assess the normality of the residuals. The ratio of the final-model deviance to the model degrees of freedom was also examined. To locate differences among the six treatment groups, pregnancy rates were analyzed by Chi-square test. Differences in fatty acid composition between oilseeds were determined with a Student's t-test.

6.4 Results

Pregnancy rate was not affected by treatments given at CIDR insertion (Table 1). However, pregnancy rates in heifers given ECP 24 h after CIDR removal (ECP24) was higher ($P<0.005$) than in those given ECP at CIDR removal (ECP0) or GnRH at TAI (GnRH52). Pregnancy rates following TAI were not influenced by location (53.4 and 57.9% for Locations A and B, respectively, $P=0.20$) or by diet (52.1, 57.0 and 57.7% for control, flax seed and sunflower seed, respectively, combined for both locations; $P=0.46$). Overall pregnancy rate for AI (TAI and reinsemination) was 79.8% (785/983) and fall pregnancy rate was 95.9% (943/983; Table 6.1).

The chemical composition of the oil seeds is presented in Table 6.2. The flax seed and sunflower seed included in the diets had a crude fat content of 34.5 and 35.3% and a dry matter content of 91.9 and 92.2%, respectively. There were profound differences in the proportion of linolenic acid (C18:3; 53.1 versus 0.18%) and linoleic acid (C18:2; 17.5 versus 70.9%) in flax seed and sunflower seed, respectively.

Table 6.1. Pregnancy rates to fixed-time AI, after rebreeding, and fall pregnancy rates in beef heifers receiving a CIDR and given 100 μg GnRH or 1 mg estradiol cypionate (ECP) and 50 mg Progesterone 5% (to synchronize follicular wave emergence), and subsequently given 0.5 mg ECP at CIDR removal (ECP0) or 24 h later (ECP24), or 100 μg GnRH concurrent with AI (GnRH52) to synchronize ovulation.

	GnRH			ECP		
	ECP0	ECP24	GnRH52	ECP0	ECP24	GnRH52
No. heifers	162	165	165	160	165	166
Fixed-time AI [1]						
No. pregnant	78	109	89	90	107	80
Pregnancy rate (%)	48.1[a]	66.1[b]	53.9[a]	56.2[ab]	64.8[b]	48.2[a]
Rebreeding [1]						
No. pregnant	47	31	40	41	31	42
Pregnancy rate (%)[2]	77.2	84.8	78.2	81.9	83.6	73.5
Fall pregnancy examination [3]						
No. pregnant	152	159	159	158	157	158
Pregnancy rate (%)	93.8	96.4	96.4	98.8	95.2	95.2

[ab] Percentages without a common superscript differ (P<0.001); ECP/ECP0 tended to differ from GnRH/ECP24 (P<0.07) and ECP/ECP24 (P<0.1)
[1] Pregnancy rate based on ultrasonography
[2] Accumulated pregnancy rate after two inseminations; percentages tended to differ (P<0.09).
[3] Pregnancy rate based on rectal palpation

Table 6.2. Chemical composition of the oil seeds used.

End point	Flax seed	Sunflower seed
Dry matter (%)	91.9	92.2
Crude protein (%)	24.3	15.1
Crude fibre (%)	8.2	21.2
Crude fat (%)	35.3	34.5
Fatty acid (% of total lipid)		
C16:0	6.3[a]	7.0[b]
C18:0	4.2[a]	5.1[b]
C18:1	18.3[a]	15.3[b]
C18:2 (Linoleic)	17.5[a]	70.9[b]
C18:3 (Linolenic)	53.1[a]	0.18[b]
C20:0	0.17[a]	0.46[b]
C20:1	0.20[a]	0.12[b]
C20:3	0.16[a]	0.70[b]
C24:0	0.11[a]	0.20[b]

[ab] Within a row, means with different superscripts are different ($P < 0.01$).

6.5 Discussion

Pregnancy rate was not significantly affected by treatments given at CIDR insertion to synchronize follicular wave emergence (55.7 versus 56.4% for heifers that received GnRH versus ECP, respectively). Treatment with GnRH induces acute release of both LH and FSH; it is speculated that if the dominant follicle present at treatment has expressed LH receptors, it will ovulate. In that regard, dominant follicles acquire LH receptors at the time of selection, approximately 3 d after the emergence of a follicular wave, when the dominant follicle achieves a diameter of 8.5 mm (Xu et al., 1995; Ginther et al., 1996a). However, ovulation did not occur consistently in heifers expected to have a dominant follicle > 10 mm diameter at the time of treatment (Martínez et al., 2003b). Therefore, there may be other factors that determine whether ovulation occurs in response to GnRH treatment. It is noteworthy that treatment with GnRH synchronizes emergence of a new follicular wave only if ovulation occurred (Martínez et al., 1999), and ovulation occurred in only 55.6% of GnRH-treated heifers in that study (Martínez et al., 1999). However, since follicular waves emerge approximately every 7 to 10 d (Ginther et al., 1989a), spontaneous emergence of a new wave (in the absence of ovulation) would occur at approximately the same time as the emergence of a new wave following ovulation in some heifers.

In contrast to GnRH, E-17β has been shown to synchronize follicular wave emergence in the majority of cattle, regardless of the stage of the follicular wave when treatment is administered (Adams et al., 1995; Bó et al., 1994). The mechanism

responsible for estrogen-induced synchronization of follicular growth appears to be systemic rather than local (Bó et al., 2000) and to involve suppression of plasma FSH concentrations, followed by synchronous resurgence of FSH (Bó et al., 1994).

Although there was no difference in pregnancy rates following TAI between heifers that received GnRH versus ECP in the present study, both treatments have been previously shown to be less efficacious than E-17β in synchronizing follicular wave emergence (Thundathil et al., 1999; Martínez et al., 2001; Colazo et al., 2003b, Chapter 5). In cattle fed melengestrol acetate in a previous study, the interval from PGF to estrus was shorter and less variable (Martínez et al., 2001), and pregnancy rate was higher (Thundathil et al., 1999) in those given E-17β compared to those given GnRH. In addition, when beef heifers in a CIDR-based program were given either 5 mg E-17β or 1 mg ECP, those in the latter group had a longer and more variable interval from treatment to emergence of a new follicular wave (Colazo et al., 2003b, Chapter 5). Only 57.1% of heifers given 1 mg ECP at random stages of the estrous cycle had a new follicular wave 3 or 4 d later (Colazo et al., 2003b, Chapter 5).

In the present study, fertility following TAI was significantly affected by treatments to synchronize ovulation. The pregnancy rate in heifers given ECP 24 h after CIDR removal (ECP24) was higher than in those given ECP at CIDR removal or GnRH at TAI (65.4, 52.2 and 51.1%, respectively). Furthermore, pregnancy rates in heifers given ECP 24 h after CIDR removal were nearly identical when heifers were given ECP or GnRH at the time of CIDR insertion (64.8 and 66.0%, respectively). In a previous

study (Colazo et al., 2003b, Chapter 5) in which ECP was used to synchronize wave emergence, pregnancy rates were lower when ECP was given concurrent with CIDR removal than 24 h later. However, when 5 mg E-17β was used to synchronize wave emergence, the timing of ECP treatment (0 or 24 h following CIDR removal) did not significantly affect the interval to the LH surge, ovulation time, or pregnancy rate following TAI (Colazo et al., 2003b, Chapter 5).

In estradiol-based regimens, administration of 0.5 to 1 mg of estradiol benzoate following progestin withdrawal and PGF treatment in cattle enhanced estrus detection, synchrony of ovulation, and pregnancy rates following TAI (Colazo et al., 1999; Ryan et al., 1995; Hanlon et al., 1996; Fike et al., 1997). Although administration of estradiol 24 h after CIDR removal requires one additional handling compared to giving estradiol at CIDR removal or GnRH treatment concurrent with AI, this was offset by higher pregnancy rates in the present study. In an Ovsynch® protocol, ovulation occurred between 24 and 34 h after the second GnRH injection (Pursley et al., 1995), and numerically higher pregnancy rates were achieved when TAI was done 8 to 24 h after GnRH treatment (Pursley et al., 1998). However, similar pregnancy rates have been recently reported in beef cows synchronized with an Ovsynch® protocol as compared to those receiving the second GnRH treatment concurrently with AI (Small et al., 2001; Geary et al., 2001). In GnRH-based protocols, the second GnRH treatment is intended to synchronize ovulation in a majority of treated cattle. However, in the present study, 17 heifers that received GnRH to synchronize ovulation were observed in estrus 4 d after TAI, suggesting that GnRH treatment did not induce ovulation in these heifers. We

speculate that the dominant follicle failed to ovulate in response to GnRH treatment (because of asynchronous wave emergence and a small dominant follicle without LH receptors) and the heifers ovulated subsequent to a delayed estrus.

At a feeding rate of 1 kg/heifer/day of oil seeds, the added quantities of crude fat, linoleic acid and linolenic acid were 353, 62 and 187 g, respectively, for flax seed, and 345, 244 and 0.6 g for sunflower seed. Although the flax seed diet had a significantly greater linolenic acid content than the other two diets, its effect on fertility was not clearly demonstrated. In studies with lactating dairy cattle, Petit et al. (2001) and Ambrose et al. (2002) reported significant improvements in pregnancy rates in cows fed a flax seed-supplemented ration. In both studies flax seed was processed in some manner before feeding to cattle. In one study (Petit et al., 2001), flax seed was treated with formaldehyde to protect the fatty acids from undergoing biohydrogenation in the rumen, whereas in the other study (Ambrose et al., 2002), both flax seed and sunflower seed were rolled. Khorasani and Kennelly (1994) reported a significantly higher level of linolenic acid in milk of cows fed rolled compared to whole flax seed.

In the present study, both oilseeds were fed unprocessed. Perhaps processing the oilseeds would have resulted in higher pregnancy rates. Furthermore, a higher level of oilseed feeding may have been more effective in improving pregnancy rates. Petit et al. (2001) included flax seed in the diet of dairy cows at 17% of the dry matter, whereas oilseeds were fed at an estimated 11% of dry matter in the present study, which is similar to that fed to dairy cows (10% of DM) in a recent study (Ambrose et al., 2002). Since

feeding flax seed at 10% (on a dry matter basis) significantly increased both pregnancy rate (Ambrose et al., 2002) and the α-linolenic acid content of milk in lactating dairy cows (Ambrose, unpublished data), we expected that feeding oilseeds at the rate of 1.0 kg/heifer/day would have the desired effect on pregnancy rates. It may be noteworthy that the pregnancy rate in dairy cows fed a control diet in the earlier study (Ambrose et al., 2002) was much lower than the pregnancy rate in beef heifers fed a control diet in the present study (32.5 versus 52.1%, respectively); an increase in pregnancy rates is more likely when fertility is low to moderate in the control group.

In summary, pregnancy rate following TAI was not significantly affected by the treatment given at CIDR insertion (100 μg GnRH versus 1 mg ECP plus 50 mg progesterone). However, 0.5 mg ECP given 24 h after CIDR removal resulted in a significantly higher pregnancy rate than 0.5 mg ECP given at CIDR removal or 100 μg GnRH given at TAI 52 h after CIDR removal. Although there was no significant effect of oilseeds on fertility, processing the oilseeds and (or) feeding higher levels of oilseed may be required. Further studies are required to verify and extend these findings.

7.0 EFFECT OF ESTRADIOL VALERATE ON OVARIAN FOLLICLE DYNAMICS AND SUPEROVULATORY RESPONSE IN PROGESTIN-TREATED CATTLE

7.1 Abstract

Three experiments evaluated the effects of estradiol valerate (EV) on ovarian follicular and CL dynamics, intervals to estrus and ovulation, and superovulatory response in cattle. Experiment 1 compared the efficacy of two norgestomet ear implants (Crestar^{TM} and Syncro-Mate B; SMB^{TM}) for 9 d (with PGF at implant removal), combined with either 5 mg estradiol-17β and 100 mg progesterone (EP) or 5 mg EV and 3 mg norgestomet (EN) im at the time of implant insertion on CL diameter and follicular wave dynamics. Ovaries were monitored by ultrasonography. There was no effect of norgestomet implant. Diameter of the CL decreased following EN treatment ($P<0.01$). Mean (± SD) day of follicular wave emergence (FWE) was earlier ($P<0.0001$) and less variable ($P<0.0001$) in EP- (3.6 ± 0.5 d) than in EN- (5.7 ± 1.5 d) treated heifers. Intervals from implant removal to estrus ($P<0.001$) and ovulation ($P<0.01$) were shorter in EN- (45.7 ± 11.7 and 74.3 ± 12.6 h, respectively) than in EP- (56.4 ± 14.1 and 83.3 ± 17.0 h, respectively) treated heifers. Experiment 2 compared the efficacy of EP versus EN in synchronizing FWE for superovulation in SMB-implanted cows. At random stages of the estrous cycle, Holstein cows (n=78) received two SMB^{TM} implants (Day 0) and

were randomly assigned to receive EN on Day 0 or EP on Day 1. Folltropin-V treatments were initiated on the evening of Day 5, with PGF in the morning and evening of Day 8, when SMB^{TM} were removed. Cows were inseminated after the onset of estrus and embryos were recovered 7 d later. Non-lactating cows had more CL (16.7 ± 11.3 versus 8.3 ± 4.9) and total ova/embryos (14.7 ± 9.5 versus 7.9 ± 4.6) than lactating cows ($P<0.05$). EP-treated cows tended ($P=0.09$) to yield more transferable embryos (5.6 ± 5.2) than EN-treated cows (4.0 ± 3.7). Experiment 3 compared the effect of dose of EV on ovarian follicle and CL growth profiles and synchrony of estrus and ovulation in CIDR-treated beef cows (n=42). At random stages of the estrous cycle (Day 0), cows received a CIDR and no further treatment (Control), or an injection of 1, 2, or 5 mg im of EV. On Day 7, CIDR were removed and cows received PGF. Follicular wave emergence occurred within 7 d in 7/10 control cows and 31/32 EV-treated cows ($P<0.05$). In responding cows, interval from treatment to FWE was longer ($P<0.05$) in those treated with 5 mg EV (4.8 ± 1.2 d) than in those treated with 1 mg (3.2 ± 0.9 d) or 2 mg (3.4 ± 0.8 d) EV, while control cows were intermediate (3.8 ± 2.0 d). Diameter of the dominant follicle was smaller ($P<0.05$) at CIDR removal and tended ($P=0.08$) to be smaller just prior to ovulation in the 5 mg EV group (8.5 ± 2.2 and 13.2 ± 0.6 mm, respectively) than in the control (11.8 ± 4.6 and 15.5 ± 2.9 mm, respectively) or 1 mg EV (11.7 ± 2.5 and 15.1 ± 2.2 mm, respectively) groups, with the 2 mg EV group (10.7 ± 1.5 and 14.3 ± 1.7 mm, respectively) intermediate. Diameter of the dominant follicle at CIDR removal was less variable ($P<0.01$) in the 2 and 5 mg EV groups than in the control group, and intermediate in the 1 mg EV group. In summary, treatment with 5 mg EV resulted in a longer and more variable interval to follicular wave emergence than treatment with 5 mg

estradiol-17β, which affected preovulatory dominant follicle size following progestin removal, and may have also affected superstimulatory response in Holstein cows. Additionally, 5 mg EV appeared to induce luteolysis in heifers, reducing the interval to ovulation following norgestomet removal. Conversely, intervals to, and synchrony of, follicular wave emergence, estrus and ovulation following treatment with 1 or 2 mg EV suggested that reduced doses of EV may be more useful for the synchronization of follicular wave emergence in progestogen-treated cattle.

7.2 Introduction

Norgestomet-based programs have been developed to synchronize estrus in cattle; estrus is suppressed in the presence of a norgestomet implant, but occurs relatively synchronously following implant removal (Odde, 1990). Two types of norgestomet-releasing ear implants have been used in estrus synchronization programs; Syncro-Mate B (SMB^{TM}) is a hydron implant containing 6 mg norgestomet, while Crestar^{TM} is a silicon implant containing 3 mg norgestomet. Treatment protocols with both types of implants include an injection of 5 mg estradiol valerate (EV) and 3 mg norgestomet at the time of implant insertion to stimulate uterine-induced luteolysis (Wiltbank et al., 1961), with implant removal after 9 d, followed by estrus detection and AI (Kastelic et al., 1999).

Exogenous estradiol has been shown to induce follicular atresia by suppressing circulating FSH levels (Price and Webb, 1988; Bó et al., 2000; Martínez et al., 2003a) shortly thereafter, a resurgence in FSH secretion occurs and a new follicular wave emerges 1 d later. The administration of 5 mg estradiol-17β in progestogen-implanted cattle was followed by the emergence of a new follicular wave 4.3 ± 0.2 d later, with little variability, regardless of the phase of follicular development at the time of treatment (Bó et al., 1995a,b). In other studies, a dose of 5 mg EV (Bó et al., 1993; Duffy et al., 2004) or estradiol benzoate (EB; Caccia and Bó et al., 1998), or 2 mg estradiol cypionate (ECP; Thundathil et al., 1997) suppressed ovarian follicle growth, but the interval to emergence of a new follicular wave was somewhat prolonged and quite variable. However, intervals to peak plasma FSH concentrations and follicular wave emergence were earlier and less variable in beef heifers treated with reduced doses of EB (Caccia and Bó, 1998; Martínez et al., 2002a, 2003a). In addition, reduced doses of EB (Martínez et al., 2000a; 2002c) or ECP (Colazo et al., 2003b; Colazo et al., 2004a; Chapters 5.0 and 6.0) have been used successfully in estrus synchronization regimens. The effects of reduced doses of EV on follicular wave dynamics have apparently not been investigated.

The objectives of this study were to compare: 1) The effects of two different norgestomet ear-implants combined with two different injectable estrogen/progestogen hormonal treatments on the synchrony of follicular wave emergence (FWE), estrus and ovulation in beef heifers (Experiment 1); 2) The efficacy of estradiol valerate plus norgestomet with estradiol-17β plus progesterone in synchronizing ovarian FWE for superstimulation of SMB-implanted cows (Experiment 2); and 3) The effects of different

doses (1, 2 and 5 mg) of estradiol valerate on ovarian follicle dynamics, CL development and intervals to FWE, estrus and ovulation in CIDR-treated beef cows (Experiment 3).

7.3 Materials and Methods

7.3.1 Experiment 1

Seventy-six Simmental-crossbred heifers, 18 to 20 mo of age, weighing between 380 and 520 kg, were assigned randomly at random stages of the estrous cycle and in two replicates to four treatment groups in a 2 x 2 factorial design (19 heifers/group). On Day 0 (beginning of the experiment), heifers received either silicone (Crestar^{TM}, Intervet Canada Ltd, Whitby, ON, Canada) or hydron (SMB^{TM}; Syncro-Mate B, Sanofi Animal Health, Overland Park, KS, USA) ear implants that contained 3 and 6 mg of norgestomet, respectively, and were subdivided to receive an im injection of either 5 mg estradiol-17β and 100 mg progesterone (EP; Sigma Chemical Co, St. Louis, MO, USA) in oil or 5 mg estradiol valerate and 3 mg norgestomet (EN; Intervet Canada Ltd and Sanofi Animal Health). On Day 9, implants were removed and all heifers received 500 μg of cloprostenol (PGF; Estrumate, Schering-Plough Animal Health, Pointe-Claire, PQ, Canada).

Heifers were monitored daily by ultrasonography (Aloka SSD 500 with a 7.5 MHz linear-array transducer; ISM Inc., Edmonton, AB, Canada) to determine ovarian follicle regression and wave emergence and dominant follicle growth, and twice daily

(starting 48 h after implant removal) to determine the time of ovulation. The diameter of the CL and all follicles ≥ 3 mm were measured ultrasonographically (Pierson and Ginther, 1984). The day of emergence of a follicular wave was defined as the day that the dominant follicle was first identified at a diameter of 4 mm (Ginther et al., 1989b). When FWE did not occur during the observation period, day of FWE, prior to treatment, was estimated from the size of the dominant follicle at the time of treatment. Ovulation was confirmed by the disappearance of a large (>10 mm) follicle that had been detected at the previous examination. Estrus detection was performed by visual observation twice daily for 40 min each.

7.3.2 Experiment 2

Lactating (n = 63) and non-lactating (n = 15) Holstein cows at random stages of the estrous cycle received two SMB^{TM} implants (Sanofi Animal Health) on Day 0 and were randomly assigned to receive 5 mg EV and 3 mg norgestomet (EN; Sanofi Animal Health) im on Day 0, or 5 mg estradiol-17β and 100 mg progesterone (EP; Sigma Chemical Co) im on Day 1. Superstimulatory treatments (400 mg NIH-FSH-P1 of Folltropin-V; Bioniche Animal Health Inc, Athens, GE, USA) in twice daily decreasing doses (80, 60, 40, and 20 mg) over a 4-d period were initiated on the evening of Day 5 (anticipated time of follicular wave emergence). Cows received PGF (Schering-Plough Animal Health) in the morning and evening of Day 8 and implants were removed in the evening of Day 8. Cows were artificially inseminated 12, 24 and 36 h after the onset of estrus, and 7 d later, ova/embryos were recovered nonsurgically by a single technician.

Body condition scores (BCS; Edmonson et al., 1989) and numbers of lactations were recorded. Numbers of CL and unovulated follicles were determined by rectal palpation on the day of ova/embryo collection, and the numbers of ova/embryos, unfertilized ova and transferable embryos collected were recorded for each cow.

7.3.3 Experiment 3

Non-lactating, crossbred beef cows (n = 42), 3 to 9 yr of age received a progesterone-releasing vaginal insert (CIDR; Bioniche Animal Health; Belleville, ON, Canada) at random stages of the estrous cycle (designated Day 0). Cows were allocated randomly to one of four groups to receive no further treatment (Control; n = 10), an im injection of canola oil containing 1 mg (n = 11), 2 mg (n = 10), or 5 mg (n = 11) EV (Sigma Chemical Co) at the time of CIDR insertion. On Day 7, CIDR were removed and PGF (Schering-Plough Animal Health) was administered. Ovaries were examined by transrectal ultrasonography (as in Experiment 1) once daily from CIDR insertion (Day 0) to 48 h after CIDR removal (Day 9) to monitor ovarian dynamics, and thereafter, twice daily to determine the time of ovulation. The day of emergence of a follicular wave was defined as in Experiment 1. The interval from FWE to ovulation was also calculated for each animal. Estrus detection was performed by visual observation three times daily for 4 d, from 24 h after CIDR removal. A responding cow was defined as having FWE within 6 d (Days 1 to 7) after CIDR insertion.

7.3.4 Statistical Analyses

Data throughout this study are reported as means ± SD. All data were analyzed using a statistical computer software program (SAS v.8.2 for Windows; SAS Institute, Cary, North Carolina, USA). In Experiments 1 and 3, measurements of central tendency and variability in follicular wave emergence between treatment groups are displayed in Box and Whisker plots (Figures 1 and 3). Intervals from treatment to FWE, from FWE to ovulation, and from CIDR removal to estrus and ovulation, and diameters of the dominant follicle at progestin removal and prior to ovulation, were analyzed using ANOVA. The initial statistical model included animal, replicate (Experiment 1) and treatment (norgestomet-implant and estradiol preparation, Experiment 1, or EV dose, Experiment 3). As replicate and norgestomet implant were found to have no significant effect, the new statistical model included animal and estradiol treatment. Means were compared with the protected LSD test and equality of variances in intervals to FWE, estrus, and ovulation were compared by Bartlett's test. In Experiment 3, the number of follicles was analyzed using generalized estimating equations (GEE; PROC GENMOD). The main-effects model was assessed for first-order interactions where treatment and day remained in the model with $P<0.05$ for both variables in the GEE analysis. Data for CL diameter were analyzed by the Proc Mixed Model procedure in SAS using autoregressive-1 (AR-1) as the covariate structure for repeated measurements (Litell et al., 1998). In Experiment 2, numbers of CL and unovulated follicles, unfertilized ova, transferable embryos and total ova/embryos were analyzed using GEE. Model specifications included a poisson distribution, logit link function, repeated statement with

subject equal to animal, and an exchangeable correlation structure. The statistical model included animal, body condition score (<2.5 or ≥2.5), number of lactations (one or ≥2), lactational status (lactating versus non-lactating), and treatment. Body condition score and number of lactations were found to have no significant effect and were excluded from the revised statistical model. Variables remaining in the final multivariable model at $P<0.05$, based on the robust empirical standard errors produced by the GEE analysis, were considered statistically significant. Proportional data were compared by Chi-square test.

7.4 Results

7.4.1 Experiment 1

One heifer (Crestar™/EP) lost her implant, one heifer (SMB™/EP) had two follicular waves during the treatment period, and another heifer (Crestar™/EP) was injured; data from these three heifers were removed from all analyses. Three heifers (two in the Crestar™/EP and one in the SMB™/EN group) did not ovulate within 6 d following implant removal. Replicates did not differ (P=0.7), and there was no effect of implant on the interval to follicular wave emergence (P=0.6) or from implant removal to estrus and ovulation (P=0.8). Follicular wave emergence occurred earlier (P<0.0001) and the interval was less variable (P<0.0001) in EP-treated (3.6 ± 0.5 d) than in EN-treated (5.7 ± 1.5 d) heifers (Figure 7.1), while the interval from implant removal to estrus was shorter (P<0.001) and tended to be less variable (P=0.09) in EN- (45.7 ± 11.7 h) than in

EP- (56.4 ± 14.1 h) treated heifers. The interval from implant removal to ovulation was also shorter ($P<0.01$) and less variable ($P<0.01$) in the EN- (74.3 ± 12.6 h) than in the EP- (83.3 ± 17.0 h) treated heifers (Figure 7.2). Diameters of dominant follicle at implant removal and just prior to ovulation were not affected by implant treatment ($P=0.8$ and 0.2, respectively). However, diameters of the dominant follicle at implant removal and just prior ovulation were greater ($P<0.02$ and 0.001) in heifers treated with EP (11.6 ± 2.0 and 13.9 ± 1.6 mm, respectively) than in those treated with EN (10.3 ± 2.6 and 12.5 ± 2.1 mm, respectively); variances for preovulatory dominant follicle size tended to differ ($P=0.08$). Diameter of the CL was affected by estradiol treatment ($P<0.01$), day ($P<0.0001$) and treatment by day interaction ($P<0.0001$), but there was no effect of implant ($P=0.55$). The CL diameter was smaller in EN- than in EP-treated heifers from Day 4 ($P<0.02$) to Day 9 ($P<0.0001$) after treatment.

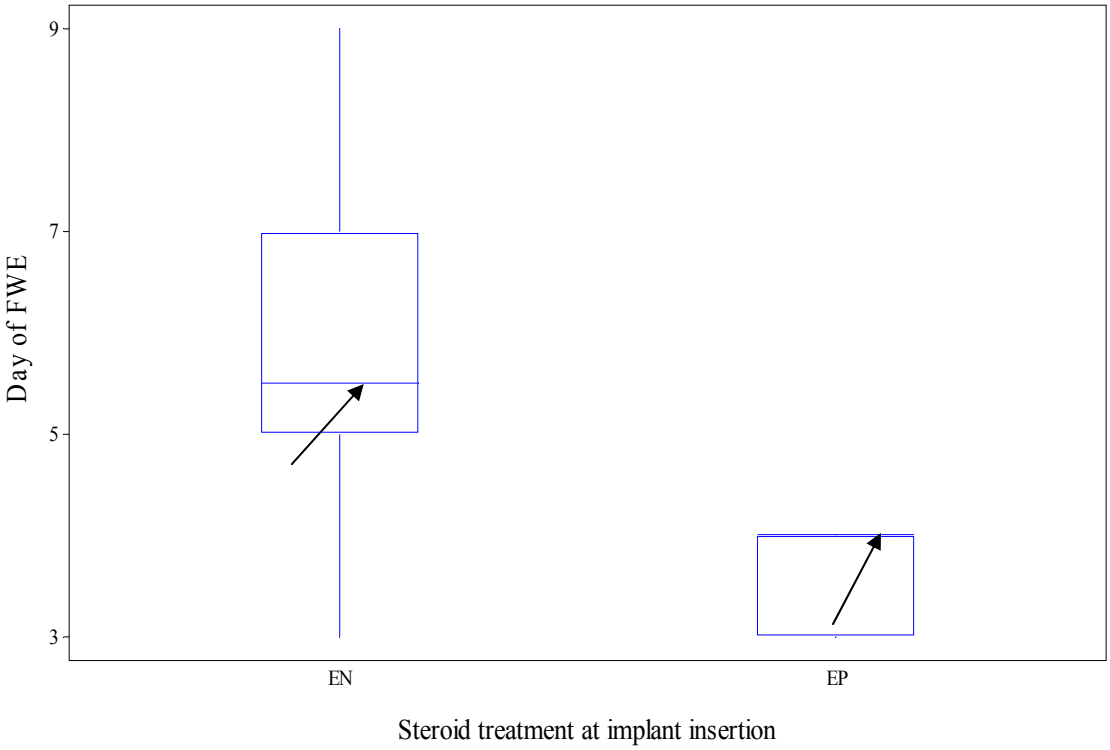

Figure 7.1. Box and Whisker plots for day of follicular wave emergence (FWE) in heifers treated with either 5 mg estradiol valerate and 3 mg norgestomet (EN; n = 38) or 5 mg estradiol-17β and 100 mg progesterone (EP; n = 35) at the time of norgestomet implant insertion. The box encloses the middle fifty-percentile of the data and is bisected by a line at the value for the median (arrows). The vertical lines (whiskers) indicate the range of data values. Follicular wave emergence occurred earlier ($P<0.0001$) and the interval was less variable ($P<0.0001$) in EP- than in EN-treated heifers.

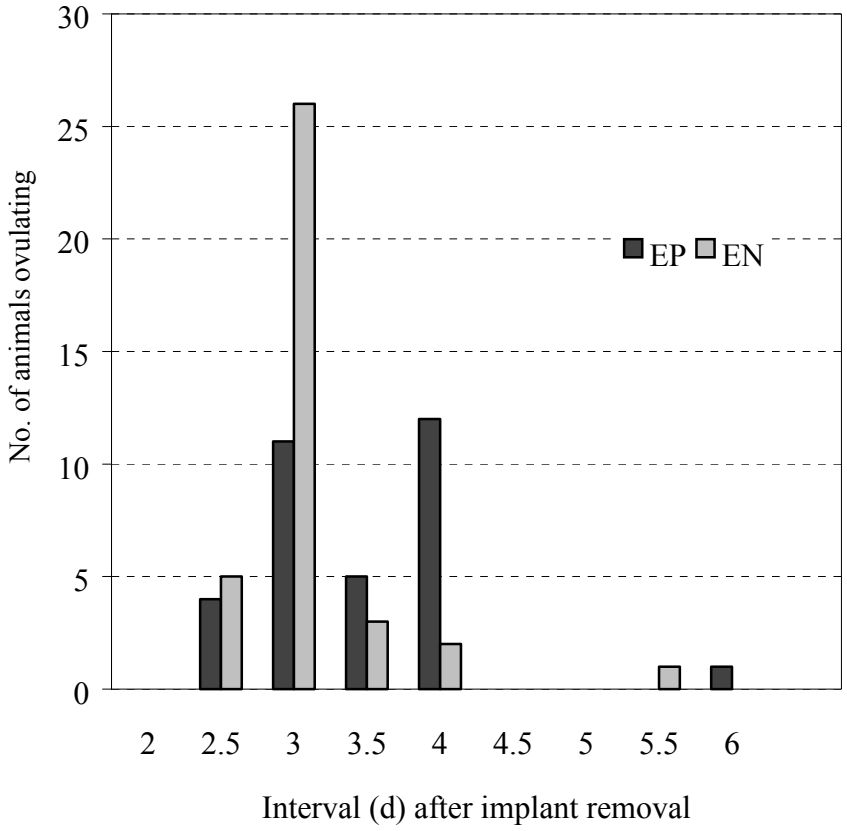

Figure 7.2. Numbers of heifers ovulating at 12 h intervals following norgestomet implant removal after treatment with either 5 mg estradiol-17β and 100 mg progesterone (EP; n= 33) or 5 mg estradiol valerate and 3 mg norgestomet (EN; n = 37) at implant insertion. The interval from implant removal to ovulation was shorter ($P<0.01$) and less variable ($P<0.01$) in the EN- than in the EP-treated heifers.

7.4.2 Experiment 2

Ten cows were not detected in estrus and were not inseminated (three cows in the EP group and seven cows in the EN group); data from these cows were removed from all analyses. Neither body condition score (overall mean of 2.9 ± 0.7), nor number of lactations (overall mean of 3.7 ± 1.6) affected superovulatory response (P=0.6). Results by lactational status and estradiol/progestogen treatment are shown in Table 7.1. There were fewer CL (8.3 ± 4.9 versus 16.7 ± 11.3; P<0.02) and total ova/embryos collected (7.9 ± 4.6 versus 14.7 ± 9.5; P<0.02) in lactating than in non-lactating cows. The number of transferable embryos tended to be higher (P=0.09) in EP-treated cows (5.6 ± 5.2) than in EN-treated cows (4.0 ± 3.7).

Table 7.1. Mean number of CL, unovulated follicles, total ova/embryos collected, unfertilized ova, degenerate and transferable embryos following superstimulation in lactating (L) and non-lactating (NL) Holstein cows treated with either 5 mg estradiol-17β and 100 mg progesterone (EP) on Day 1 or 5 mg estradiol valerate and 3 mg norgestomet (EN) on Day 0 (SMB™ implant insertion).

	L/EP	L/EN	NL/EP	NL/EN
No. cows	29	26	7	6
CL	9.2[b]	7.2[b]	17.4[a]	15.8[a]
Range	2 to 20	2 to 30	6 to 30	2 to 30
Unovulated follicles	0.9	1.0	1.3	2.2
Range	0 to 4	0 to 5	0 to 2	0 to 5
Total ova/embryos	9.4[a]	6.3[a]	15.1[b]	14.2[b]
Range	2 to 19	1 to 19	5 to 30	2 to 27
Unfertilized ova	3.0[a]	1.3[a]	6.8[b]	5.6[b]
Range	0 to 14	0 to 8	0 to 27	0 to 10
Degenerated embryos	1.3	1.2	0.1	1.6
Range	0 to 9	0 to 6	0 to 1	0 to 5
Transferable embryos	5.0[cd]	3.8[d]	8.1[c]	5.5[c]
Range	0 to 18	0 to 15	3 to 27	0 to 12

[ab] Means within a row with different superscripts differed significantly ($P<0.05$)

[cd] Means within a row with different superscripts tended to differ ($P=0.1$)

7.4.3 Experiment 3

One cow (5 mg EV group) lost her CIDR, and all data from this animal were excluded from analyses. A new follicular wave did not emerge during the observation period in 3 of 10 control cows. Overall, 96.7% (30/31; $P<0.05$) of EV-treated cows had a new follicular wave within a range of 6 d (Days 1 to 7) following treatment (Figure 7.3). In responding cows, interval from treatment to wave emergence was longer ($P<0.05$) in those treated with 5 mg EV (4.8 ± 1.2 d) than in those treated with 1 mg (3.2 ± 0.9 d) or 2 mg (3.4 ± 0.8 d) EV, while control cows were intermediate (3.8 ± 2.0 d). Intervals to follicular wave emergence (d) and from CIDR removal to estrus and ovulation (h) in all treated cows are shown in Table 7.2. Four cows ovulated between Days 0 and 2 of the experiment (three from the 2 mg EV treatment group and one from the control group), and diameters of the CL (14.0 ± 3.9 mm) and dominant follicle (13.5 ± 0.6 mm) for these cows on Day 0 suggested that they were in late diestrus or early proestrus at the time of the treatment. The interval from follicular wave emergence to ovulation was shorter ($P<0.05$) in cows treated with 5 mg EV (6.1 ± 0.6 d) than those in the control group (7.7 ± 2.1 d), or those treated with 1 (7.4 ± 1.0 d) or 2 (7.4 ± 1.1 d) mg EV; the interval was more variable ($P<0.005$) in the control group than in the 1 or 5 mg EV groups, and tended ($P=0.07$) to be more variable than in the 2 mg EV group.

The number of small follicles was affected by day ($P<0.005$), but not by treatment ($P=0.7$). However, cows in the 1, 2, and 5 mg groups tended ($P=0.08$) to have more small follicles on Day 4, than those in the control group. When the numbers of small follicles

were normalized to day of follicular wave emergence (Figure 7.4), only a day effect ($P<0.001$) was observed. The number of intermediate follicles was affected by day, treatment and day by treatment interaction ($P<0.001$). Corpus luteum diameter decreased over time in all groups. There was an effect of day ($P<0.0001$) on CL diameter, but the effect of treatment was not significant ($P=0.3$), while the treatment by day interaction tended ($P=0.1$) to be significant. Diameter of the dominant follicle was smaller ($P<0.04$) at CIDR removal and tended to be smaller ($P=0.08$) just prior to ovulation in the 5 mg EV group (8.5 ± 2.2 and 13.2 ± 0.6 mm, respectively) than in the control (11.8 ± 4.6 and 15.5 ± 2.9 mm) or 1 mg EV (11.7 ± 2.5 and 15.1 ± 2.2 mm) groups; the 2 mg EV group (10.7 ± 1.5 and 14.3 ± 1.7 mm) was intermediate. Diameter of the dominant follicle at CIDR removal was less variable ($P<0.01$) in the 2 and 5 mg EV groups than in the control group, and intermediate in the 1 mg EV group.

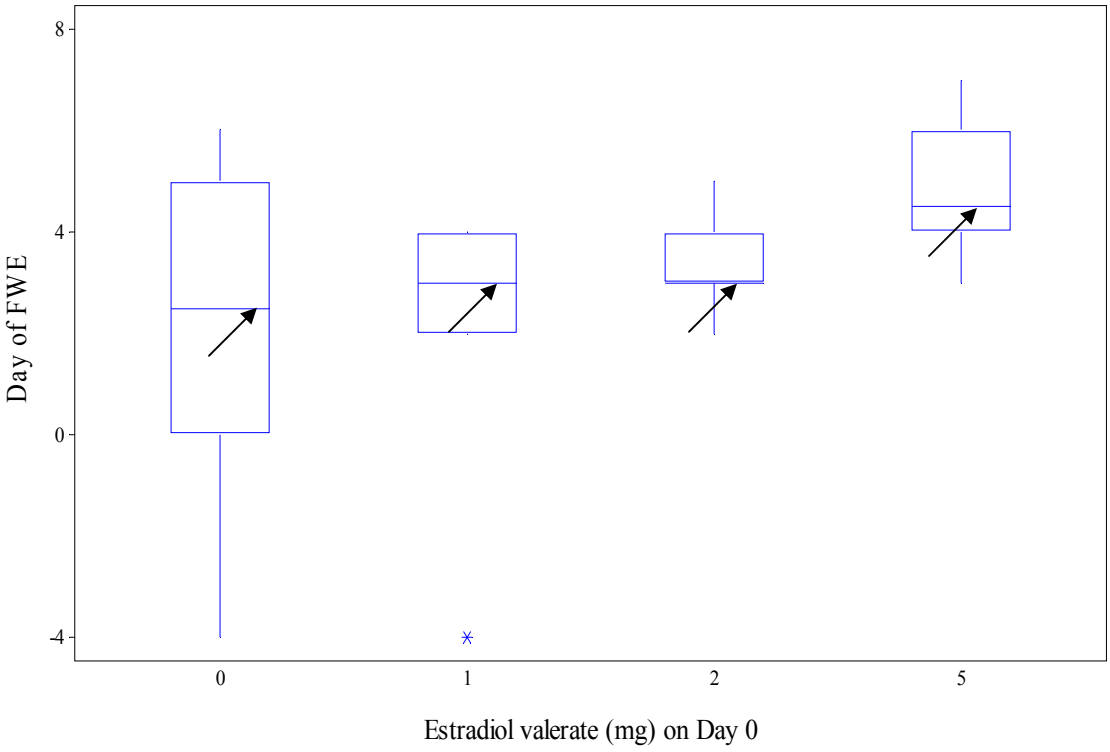

Figure 7.3. Box and Whisker plots for day of follicular wave emergence (FWE) in CIDR-treated control cows (0; n = 10) or those receiving 1 mg (n = 11), 2 mg (n = 10), or 5 mg (n = 10) estradiol valerate on Day 0 (CIDR insertion). The box encloses the middle fifty-percentile of data and is bisected by a line at the value for the median (arrows). The vertical lines (whiskers) indicate the range of data values. Treatment did not alter ovarian follicle dynamics in three control cows and one cow treated with 1 mg of estradiol valerate (* FWE was estimated to occur on Day –4 in this cow).

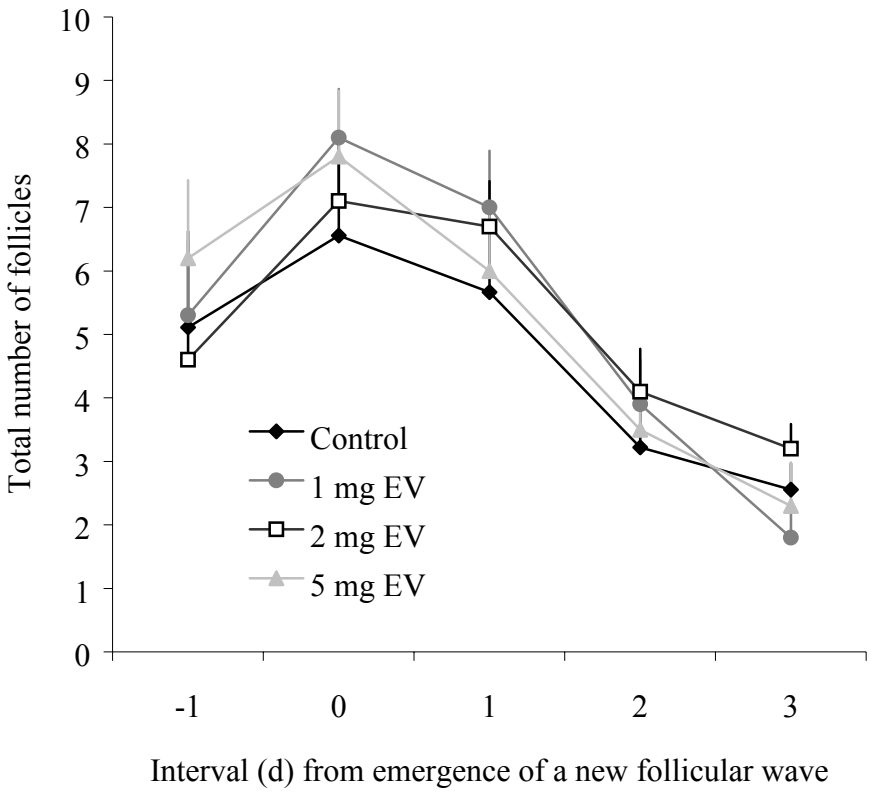

Figure 7.4. Numbers of small follicles (between 3 and 5 mm of diameter), normalized to the day of follicular wave emergence (Day 0), in beef cows treated with a CIDR and 0 (Control; n = 7), 1 mg (n = 10), 2 mg (n = 10), or 5 mg (n = 10) estradiol valerate (EV) on Day 0 (CIDR insertion). The number of follicles was affected by day ($P<0.001$) but not by treatment ($P=0.7$).

Table 7.2. Mean (± SD) intervals from treatment with 0, 1, 2 or 5 mg estradiol valerate at CIDR insertion to follicular wave emergence (FWE), and from CIDR removal to estrus and ovulation.

	Dose of estradiol valerate (mg)			
	0	1	2	5
Interval from treatment to FWE (d)				
No. cows	10	11	10	10
Mean	2.2[a]	2.5[a]	3.4[a]	4.8[b]
SD	3.3[x]	2.3[x]	0.8[y]	1.2[y]
Range	-4 to 6	-4 to 4	2 to 5	3 to 7
Interval from CIDR removal to:				
Estrus (h)				
No. cows	9	11	10	8
Mean	58.0	53.5	61.2	59.3
SD	19.4	11.2	14.1	17.1
Range	36 to 96	36 to 72	48 to 84	48 to 96
Ovulation (h)				
No. cows	10	11	10	10
Mean	86.4	85.1	91.2	90.0
SD	18.6	12.5	14.1	22.1
Range	60 to 120	72 to 108	72 to 120	72 to 132

[ab] Means within the row with different superscripts differed significantly ($P<0.05$)

[xy] SD within the row with different superscripts differed significantly ($P<0.001$)

7.5 Discussion

In this study, follicular wave dynamics following the administration of two different estradiol preparations and three different doses of an estradiol ester, estradiol valerate, were examined. Administration of 5 mg of E-17β resulted in synchronous emergence of a new follicular wave in 3.6 ± 0.5 d (Experiment 1), while administration of 5 mg EV resulted in FWE in 5.7 ± 1.5 d in heifers (Experiment 1) and 4.8 ± 1.2 d in cows (Experiment 3). In addition to the longer interval from treatment to FWE, there was considerably more variability following the administration of 5 mg EV. These findings are consistent with previous reports (Bó et al., 1993, 1995a,b), and are supported by a reduced superovulatory response following synchronization of FWE with 5 mg EV in Experiment 2.

Emergence of a new follicular wave depends on a transient increase in circulating FSH concentrations. Following administration of estradiol and progesterone, circulating FSH concentrations and LH pulse amplitude and frequency have been shown to decrease, resulting in the regression of FSH- and LH-dependent follicles (Ireland and Roche et al., 1982; Price and Webb, 1988; Bó et al., 1995b). As plasma estradiol concentrations begin to decline, a resurgence of plasma FSH concentrations occurs and emergence of a new follicular wave occurs approximately 1 d later.

Different estradiol preparations have also been shown to suppress FSH release for varying intervals of time (O'Rourke et al., 2000; Martínez et al., 2003a). Esterified estrogens (e.g. EB, EV, and ECP) are absorbed very slowly following intramuscular injection; the longer the ester chain, the lower the water solubility and the longer the interval required for estradiol to be absorbed (reviewed in Mapletoft et al., 2002a). In this regard, it has been shown that peak circulating concentrations of estradiol occurred within 2 h after an injection of 5 mg estradiol-17β, followed by a return to baseline at about 36 h (Bó et al., 2000; Martínez et al., 2003a), while administration of 5 mg of EV or EB resulted in a much more gradual rise in circulating estradiol concentrations, reaching a peak at 12 to 24 h and returning to baseline at approximately 96 h (Bó et al., 1993, Martínez et al., 2003a). In ovariectomized cows treated with 5 mg estradiol-17β, plasma FSH concentrations increased earlier and reached higher concentrations than in those treated with 5 mg EV or EB (Martínez et al., 2003a). These differences in circulating estradiol concentrations, and the delayed resurgence in plasma FSH concentrations following injection of 5 mg EV could account for the longer and more variable intervals from treatment to FWE observed in this study.

Although an injection of 5 mg EV or EB resulted in elevated circulating concentrations of estradiol for at least 96 h, FSH concentrations began to increase at about 60 h (Martínez et al., 2003a), suggesting that there may be other factors affecting the resurgence in circulating FSH concentrations (Duffy et al., 2004). However, administration of lower doses of EB resulted in highly synchronous FWE (Caccia and Bó, 1998; Moreno et al., 2001; Martínez et al., 2002a), suggesting that reduced doses of

estradiol esters can be useful for the synchronization of FWE (Caccia et al., 1998). Indeed, a dose of 1 or 2 mg EV in Experiment 3 resulted in an interval to, and synchrony of, FWE (3.2 ± 0.9 d and 3.4 ± 0.8 d, respectively) similar to that observed following administration of 5 mg estradiol-17β in Experiment 1, or reduced doses of EB in previous reports (Caccia and Bó, 1998; Moreno et al., 2001; Martínez et al., 2002a).

A dose of 1 mg EB in beef heifers resulted in FWE in 4 d and has been used successfully in estrus synchronization programs for timed AI (Martínez et al., 2001). However, Caccia and Bó (1998) reported a less variable interval to FWE after the administration of 2.5 mg EB compared to 1 mg EB in beef cows. In another study (Day et al., 2000), first service conception rates tended to be higher following administration of 2 mg EB (61.7%) in progestin-treated, lactating dairy cows, than following administration of 1 mg EB (49.0%). In Experiment 3, administration of 1 mg EV did not induce follicle regression in a cow that was in late diestrus at the time of treatment. It is not clear whether the apparent lack of response in this animal was due to the dose of EV or the stage of estrous cycle when the treatment was administered. Recent data suggest that it is difficult to induce follicle regression with estradiol and progesterone when cattle are treated late in the estrous cycle (Kastelic et al., 2004). In any case, it appears that a dose of 1 and 2 mg of either EB or EV should be recommended to synchronize FWE in estrus synchronization protocols for heifers and cows, respectively. Clearly, estradiol esters are efficacious in synchronizing FWE in cattle, providing the appropriate dose is used.

Various factors have been reported to influence the superovulatory response of cattle (Kafi and McGowman et al., 1997), including those related to ovarian status at the time of gonadotropin treatments and the timing of treatment with respect to the estrous cycle (Lindsell et al., 1986). Nasser et al. (1993) and Baracaldo et al. (2000) have shown that gonadotropin treatments must be initiated at the expected time of the follicular wave emergence in order to optimize superovulatory response. Hence, synchronization of FWE prior to initiation of superstimulatory treatments in cattle is recommended (Mapletoft et al., 2002b). In Experiment 1, heifers receiving EV had greater variability in the interval to FWE than those receiving estradiol-17β, explaining the differences in superovulatory responses in Experiment 2; Holstein cows given estradiol-17β tended to have more transferable embryos than those treated with EV. Data suggest that the reason for this difference is that FSH treatments more closely coincided with FWE in estradiol-17β-treated cows. However, data also suggest that when using estradiol esters, a 2 mg dose of EB (Caccia et al., 1998) or EV (Experiment 3), in combination with a progestin, would be efficacious in the synchronization of FWE for superstimulation, regardless of the category of animal.

Superstimulatory response was also affected by lactational status at the time of initiation of gonadotropin treatments. Lactation is an extrinsic factor that has been shown to affect superovulatory responses of cattle (Lindsell et al., 1986). However, the effect of lactation is probably related more to level of nutrition and energy balance within individual animals. In this regard, a negative energy balance during lactation has been shown to be associated with decreased LH pulse frequency, and decreased growth rates

and diameters of dominant follicles (Roche et al., 2000). It has also been hypothesized that follicles growing under these circumstances may contain oocytes of inferior quality (Britt, 1992). In the current study, non-lactating cows had more CL, total ova/embryos and transferable embryos than lactating cows. However, the numbers of transferable embryos seemed to be affected more by superovulatory response than oocyte/embryo quality.

Estrogens are involved in natural luteolysis, and the luteolytic effects of exogenously administered estrogens have been shown to be mediated through uterine release of PGF (Hixon et al., 1983; Peterson et al., 2000). Although mean CL diameter was reduced 24 and 48 h after treatment with 5 mg estradiol-17β in this study, it subsequently returned to pre-treatment values and was maintained until implant removal. In contrast, the reduction in diameter of the CL following treatment with 5 mg EV was sustained and at implant removal CL diameter was significantly smaller than in the estradiol-17β-treated group. These findings confirm the luteolytic effect of estradiol esters in cattle (Wiltbank et al., 1961; Scaramuzzi et al., 1974; Munro and Moore, 1985); plasma progesterone concentrations decreased from 2 to 7 d after treatment with 5 mg EB, depending on stage of the estrous cycle when treatment was initiated (Munro and Moore, 1985). However, the luteolytic effects of estradiol esters would also appear to be dose related; a decreased CL diameter was clearly evident 4 d after treatment with 5 mg of EV in Experiments 1 and 3, but there was no evidence that doses of 1 or 2 mg EV affected luteal function in Experiment 3.

Following implant removal, heifers treated with 5 mg EV had a shorter interval to estrus and ovulation than those treated with 5 mg estradiol-17β. Although it has been shown that the interval to ovulation is affected by the stage of development of the dominant follicle at the time of PGF treatment (Kastelic et al., 1990a; Colazo et al., 2002b), EV-treated heifers had a smaller dominant follicle at time of PGF treatment (and implant removal) in the present study. The interval to estrus and ovulation may have been influenced by the low circulating progesterone concentrations at the time of implant removal, or the prolonged circulating blood levels of estradiol following the administration of 5 mg EV. In another study in ovariectomized cows (Larson and Kiracofe, 1995), plasma estradiol concentrations were found to remain elevated for approximately 10 d following injection of 5 mg EV, and behavioural estrus was detected shortly after norgestomet implant removal on Day 9. In a more recent study (Martínez et al., 2003a), 5 of 6 ovariectomized cows treated with 5 mg EV at CIDR insertion showed behavioural estrus within 12 h after CIDR removal on Day 7.

Interestingly, EV-treated heifers also ovulated more synchronously than those treated with estradiol-17β. However, heifers given 5 mg EV also had a shorter interval from FWE to ovulation; the smaller diameter of the preovulatory follicle could result in reduced fertility. Recent reports indicate that GnRH-induced ovulation of small (≤ 12 mm) dominant follicles may result in either lower fertility to timed AI (Vasconcelos et al., 2001; Colazo et al., 2004c) or increased embryonic losses (Perry et al., 2003). Therefore, fertility following progestin removal may be adversely affected by a dose of 5

mg EV compared to smaller doses of EV or 5 mg estradiol-17β in a 7 or 9 d progestin protocol.

In conclusion, estradiol treatment affected ovarian follicular dynamics; the interval from treatment to FWE in heifers was longer and more variable following treatment with 5 mg of EV than with 5 mg of estradiol-17β. The asynchrony of wave emergence following administration of 5 mg EV compared to treatment with estradiol-17β resulted in a reduced number of transferable embryos following superstimulation of Holstein cows. Treatment with 5 mg EV also resulted in luteolysis in norgestomet-implanted heifers, and a shorter interval from implant removal to estrus and ovulation. Dominant follicle diameter at progestin removal and just prior to ovulation was affected by dose and estradiol preparation. However, ovarian FWE and synchrony of estrus and ovulation in CIDR-treated cows given 1 or 2 mg EV were similar to those of norgestomet-implanted cows given estradiol-17β, suggesting that reduced doses of EV may be useful in protocols designed for superstimulation or estrus synchronization for timed AI.

8.0 FERTILITY IN BEEF CATTLE GIVEN A NEW OR PREVIOUSLY USED CIDR INSERT AND ESTRADIOL, WITH OR WITHOUT PROGESTERONE.

8.1 Abstract

The objective was to compare pregnancy rates following timed AI (TAI) in beef cattle given a new or previously used CIDR insert and injections of estradiol, with or without progesterone, to synchronize follicular wave emergence. In Experiment 1, heifers (n = 616) received a new or once-used CIDR insert for 9 d and were given 1 mg estradiol cypionate (ECP), with or without 100 mg of a commercial progesterone preparation (CP), at CIDR insertion. Heifers were treated with PGF at CIDR removal and 0.5 mg ECP im 24 h later, with TAI 55 to 60 h after CIDR removal. Pregnancy rate was not affected by either the number of CIDR uses (P=0.59; 48.3 versus 46.2% for new versus once-used CIDRs, respectively) or the addition of progesterone (P=0.42; 45.6 versus 48.8% for ECP+CP and ECP, respectively). In Experiment 2 (replicated at two locations), heifers (n = 56) and lactating beef cows (n = 307) received a once- or twice-used CIDR and an im injection of 1 mg estradiol benzoate (EB), with or without 100 mg progesterone, at CIDR insertion. Cattle received PGF in the ischiorectal fossa at CIDR removal (Day 7) and 1 mg EB im 24 h later, with TAI 52 to 56 h after CIDR removal. Pregnancy rate was affected by location (P<0.002; 46.0 versus 61.1% for Locations A and B, respectively),

parity (P<0.04; 67.9 versus 53.1% in heifers and cows, respectively) and numbers of times the CIDR had been used (P<0.03; 62.4 versus 48.4% for once- and twice-used CIDRs, respectively). However, the addition of progesterone to the injection of EB at CIDR insertion did not affect pregnancy rate (P=0.6). In Experiment 3, heifers (n = 187) received one new, one once-used, one twice-used or two twice-used CIDRs for 7 d and 2 mg EB plus 50 mg of CP at the time of CIDR insertion. Heifers were treated with PGF at CIDR removal and 1 mg EB im 24 h later, with TAI 52 to 56 h after CIDR removal. Pregnancy rate was not affected by treatments (P=0.28; 57.5, 63.8, 47.9, 47.9% for one new, one once-used, one twice-used, or two twice-used CIDRs, respectively). In summary, pregnancy rate following TAI did not differ between cattle synchronized with a new or once-used CIDR, but pregnancy rate was lower in cattle synchronized with a twice-used CIDR; however, the insertion of two twice-used CIDRs did not affect pregnancy rates. The addition of an injection of progesterone to the estradiol treatment at CIDR insertion did not enhance pregnancy rate following TAI.

8.2 Introduction

Administration of estradiol in progestin-treated cattle (Bó et al., 1995b; Caccia and Bó, 1998; Martínez et al., 2000b) has been shown to result in emergence of a new ovarian follicular wave 3 to 5 d later. However, the administration of estradiol alone to cattle without progestin implants (Bó et al., 1994) or concurrent with PGF-induced luteolysis (Burke et al., 1997b) had variable effects on follicular growth profiles, and emergence of the next follicular wave was often delayed. In addition, estradiol

concentrations in the largest follicle were suppressed following administration of estradiol benzoate and progesterone, whereas intrafollicular concentrations of estradiol were unchanged in heifers given only estradiol benzoate (Diskin et al., 2002), suggesting that follicular wave emergence may be more synchronous when progesterone was added to the synchronization protocol. However, insertion of a new CIDR for at least 24 h resulted in atresia of a persistent dominant follicle and synchronous emergence of a new follicular wave (Cavalieri et al., 1998), and the insertion of a new CIDR concurrent with administration of PGF was sufficient to facilitate estradiol-induced follicular wave emergence in cattle (Burke et al., 1998).

When ovariectomized beef cows received a once-used CIDR, a concurrent injection of 100 mg of progesterone was needed to achieve plasma progesterone concentrations (by 24 h after CIDR insertion) that were similar to those in cows treated with a new CIDR (Martínez et al., 2003a). Although the initial progesterone release by a new CIDR insert might be sufficient to induce dominant follicle regression in cattle and ensure the presence of a growing dominant follicle following CIDR removal, follicular wave emergence may be more synchronous following treatment with estradiol and progesterone, in combination, especially when a new CIDR was not used.

Many CIDR-based estrus synchronization protocols for timed AI (TAI) in cattle include an injection of estradiol and progesterone at CIDR insertion (to synchronize follicular wave emergence), with CIDR removal 7 or 8 d later. As CIDR inserts release

progesterone for at least 15 d (Macmillan et al., 1991; Macmillan and Peterson, 1993), a CIDR could potentially be used at least twice in current estrus synchronization protocols.

The objective of the present study was to determine pregnancy rates following TAI during spring breeding in western Canada in beef cattle given a new or previously used CIDR, and injections of estradiol, with or without progesterone, to synchronize follicular wave emergence.

8.3 Materials and Methods

8.3.1 Experiment 1

Angus and Angus-cross beef heifers, 12 to 15 mo of age and 280 to 400 kg of weight were managed in a drylot and fed a barley silage-based diet. At the beginning of the synchronization program, heifers were examined by transrectal ultrasonography (Aloka SSD 500 equipped with a 7.5 MHz linear-array transducer; ISM Inc., Edmonton, AB, Canada) to determine cyclicity (evidence of luteal tissue) and eliminate those with abnormalities of the reproductive tract. On Day 0, heifers at random stages of the estrous cycle received either a new (n = 300) or once-used (n = 316) controlled internal drug release vaginal insert (CIDR; Bioniche Animal Health Canada Inc., Belleville, ON, Canada) and 1 mg estradiol cypionate (ECP; Pharmacia Animal Health, Orangeville, ON, Canada) alone, or plus 100 mg progesterone (CP; Progesterone 5%; Vétoquinol N-A Inc., Lavaltrie, QC, Canada), in a two by two factorial design.

After initial use, the once-used CIDR inserts were soaked in a povidone-iodine-based detergent solution (Betadine Scrub; Purdue Pharma, Pickering, ON, Canada) for approximately 2 h, individually scrubbed with a brush, thoroughly rinsed with water, allowed top air-dry and autoclaved (at 250°C and 20 psi for 30 min), fast-dried exhaust for 20 min, wrapped in brown paper, and stored at room temperature until use. The CIDRs were inserted into the vagina as described by Macmillan et al. (1991) and the nylon filament attached to the CIDR was cut even with the vulva lips to minimize removal by pen-mates. On Day 9, CIDRs were removed and heifers received 25 mg dinoprost (Lutalyse; Pharmacia Animal Health) into the ischiorectal fossa (Colazo et al., 2002a); 24 h later, heifers received 0.5 mg ECP im.

Artificial insemination was done from 55 to 60 h after CIDR removal (Colazo et al., 2003b), using frozen-thawed semen from two different bulls, randomized with respect to treatment groups. Transrectal ultrasonography for pregnancy diagnosis was done approximately 35 d after TAI.

8.3.2 Experiment 2

Location A

Puberal crossbred beef heifers, 13 to 15 mo of age (n = 32), and lactating crossbred beef cows (n = 105), kept in an outdoor paddock under drylot conditions (fed a silage-based diet) were examined as described in Experiment 1. Body condition score

(range, 1 to 5; Houghton et al., 1990) and days postpartum were recorded for cows. On Day 0, cattle (at random stages of the estrous cycle) received a once-used (n = 66) or twice-used (n = 71), autoclaved CIDR (as in Experiment 1) and an im injection of 1 mg estradiol benzoate (EB) alone, or plus 100 mg progesterone (P; both from Sigma Chemical Co, St. Louis, MO, USA), in 2 mL canola oil, by replicate (as they came through the chute), in a two by two factorial design. All cattle received 500 μg cloprostenol (Estrumate; Schering Plough Animal Health, Pointe-Claire, QC, Canada) into the ischiorectal fossa at CIDR removal on Day 7 and 1 mg EB im 24 h later. The CIDR inserts had been previously used for 7 d (once-used) or 14 d (twice for 7 d; twice-used) prior to this study. In all cattle, TAI was done between 52 and 54 h after CIDR removal, using frozen-thawed semen from two different bulls (randomized with respect to treatment groups). Ultrasonographic pregnancy diagnosis was done (as in Experiment 1) 28 d after TAI.

Location B

Puberal Angus beef heifers (n = 24) and lactating crossbred beef cows (n = 202) managed under pasture conditions were used at this location. Body condition and postpartum intervals were recorded for cows. Cattle were examined by transrectal ultrasonography as at Location A and received a once-used (n = 115) or twice-used (n = 111), autoclaved CIDR insert for 7 d (at random stages of the estrous cycle), and estradiol and progesterone treatments as at Location A. Artificial insemination was done (by a

single inseminator) from 27.5 to 32.5 h after the second injection of EB (equivalent to 51.5 to 56.5 h after CIDR removal). Pregnancy diagnosis was done as at Location A.

8.3.3 Experiment 3

Angus and Angus-cross beef heifers (n = 187), 12 to 15 mo of age and approximately 300 to 425 kg were kept in outdoor paddocks under drylot conditions and fed a barley silage-based diet. On Day 0, heifers (at random stages of the estrous cycle) were randomly assigned to four groups to receive one new (n = 33), one once-used (n = 58), one twice-used (n = 48) or two twice-used (n = 48) CIDR inserts and 2 mg EB (Sigma Chemical Co) plus 50 mg CP (Vétoquinol N-A Inc.). The previously used CIDR inserts were washed, dried, and autoclaved before use as in Experiments 1 and 2. On Day 7, CIDRs were removed and heifers received 500 μg cloprostenol (Estrumate; Schering Plough Animal Health); on Day 8 (24 h later), heifers received 1 mg EB im. Artificial insemination was done 52 to 56 h after CIDR removal, using frozen-thawed semen from two different bulls, randomized with respect to treatment groups. Pregnancy diagnosis was done 34 to 35 d after TAI by transrectal ultrasonography.

8.3.4 Statistical Analyses

In Experiment 1, pregnancy status was analyzed with the Mantel-Haenszel test. All other qualitative comparisons in Experiments 1 and 3 were done by Chi-square procedures. In Experiment 2, the association between pregnancy status and treatment

group, location, and parity were analyzed using generalized estimating equations (GEE). Data were analyzed using a statistical computer software program (PROC GENMOD; SAS v.8.2 for Windows, SAS Institute, Cary, NC, USA). Treatments were analyzed as fixed effects with two categories; location and parity were considered as possible covariates and analyzed as categorical fixed effects. Variables remaining in the final multivariable model at $P<0.05$, based on the robust empirical standard errors produced by the GEE analysis, were considered statistically significant. The main-effects model was assessed for first-order interactions where treatment and location or parity remained in the model with $P<0.05$ for both variables in the GEE analysis. Only interactions that were $P<0.10$ are reported.

8.4 Results

8.4.1 Experiment 1

At the beginning of the experiment, 16.4% (101/616) of heifers were prepuberal (absence of luteal tissue). However, pregnancy rate did not differ ($P=0.42$) between prepuberal (43.6%) and puberal heifers (48.0%) or among treatments ($P=0.47$; overall, 47.2%, Table 8.1). A total of seven CIDR inserts (1.1%) were lost; one was new and the other six were used ($P<0.07$).

Table 8.1. Pregnancy rates after fixed-time AI in beef heifers that received a new or once-used CIDR plus an im injection of 1 mg estradiol cypionate (ECP) with (+CP) or without (-CP) 100 mg of a commercial progesterone preparation at the time of CIDR insertion.

	CIDR			
	New		Once-used	
	+CP	-CP	+CP	-CP
Heifers pregnant (n)	70/149	75/151	71/160	75/156
Pregnancy rate (%)	46.9	49.6	44.3	48.0

Pregnancy rates did not differ among treatment groups (P=0.47).

8.4.2 Experiment 2

The mean (± SEM) body condition score for cows was 2.8 ± 0.28 (range, 2 to 4) and postpartum interval was 72.3 ± 11.6 d (range, 42 to 91 d) at Location A, and 2.8 ± 0.27 (range, 2 to 3.5) and 91.5 ± 25.5 d (range, 41 to 176 d), respectively at Location B. There were effects of CIDR treatment ($P<0.03$), location ($P<0.002$), parity ($P<0.0.4$) and a location by CIDR treatment interaction ($P<0.06$) on pregnancy rates. However, pregnancy rate was not affected by body condition score ($P=0.4$) or injection of progesterone at the time of CIDR insertion ($P=0.6$).

Pregnancy results are shown in Table 8.2. Overall, cattle that were treated with a once-used CIDR had a higher pregnancy rate than those treated with a twice-used CIDR (62.4 versus 48.4%, respectively), heifers had a higher pregnancy rate than cows (67.9 versus 53.1%, respectively), and cattle at Location B had a higher pregnancy rate than those at Location A (61.1 versus 46.0%, respectively). Only cows lost CIDR inserts; the CIDR loss rate was 15.2% (16/105) at Location A and 6.9% (14/202) at Location B. Although there was no difference ($P=0.6$) in CIDR loss rate between those used once (14/30) or twice (16/30), cows that lost their CIDR inserts at Location A had a shorter ($P<0.003$) postpartum interval (67.0 ± 3.3 d) than those that did not (76.4 ± 1.1 d). Pregnancy rate was lower ($P<0.0001$) in cows that lost their CIDR inserts than those that did not (20.0 versus 56.7%, respectively).

Table 8.2. Pregnancy rates following fixed-time AI in beef cattle synchronized with a once- or twice-used CIDR plus an im injection of 1 mg estradiol benzoate with (+P) or without (-P) 100 mg progesterone at the time of CIDR insertion.

	CIDR			
	Once-used		Twice-used	
	+P	-P	+P	-P
Location A				
Cattle pregnant (n)	18/34	14/32	15/35	16/36
Pregnancy rate (%)	52.9	43.8	42.9	44.4
Location B				
Cattle pregnant (n)	42/59	39/56	31/55	26/56
Pregnancy rate (%)	71.2[a]	69.6[a]	56.4[ab]	46.4[b]
Overall				
Cattle pregnant	60/93	53/88	46/90	42/92
Pregnancy rate (%)	64.5[c]	60.2[c]	51.1[cd]	45.6[d]

[ab] Within a row, percentages without a common superscript differ (P<0.03)
[cd] Within a row, percentages without a common superscript differ (P<0.05)

8.4.3 Experiment 3

Pregnancy rates following TAI did not differ (P=0.28) among groups. Pregnancy rates were 57.5% (19/33) for one new CIDR, 63.8% (37/58) for one once-used CIDR, 47.9% (23/48) for one twice-used CIDR, and 47.9% (23/48) for two twice-used CIDR inserts.

8.5 Discussion

Although fertility following TAI was not different between cattle receiving a new or once-used CIDR, pregnancy rate was reduced in cattle receiving a twice-used CIDR in Experiment 2. These results could not be confirmed statistically with the number of animals available in Experiment 3, but pregnancy rate in heifers treated with a twice-used CIDR was numerically lower, and this was not improved by the inclusion of two twice-used CIDR inserts. The amount of progesterone released from a CIDR over a 15-d period has been reported to be highly repeatable (Macmillan et al., 1991; Macmillan and Peterson, 1993); plasma progesterone concentrations in ovariectomized cows on Days 14 and 15 were 1.9 (Macmillan et al., 1991) and 2.3 ng/mL, respectively (Peterson and Henderson, 1991), suggesting that a CIDR could be used for at least two 7-d synchronization protocols. Plasma progesterone concentrations above 1 ng/mL were expected to be sufficient to suppress the endogenous LH surge (Savio et al., 1993). However, the lower pregnancy rate observed in cattle receiving a twice-used CIDR insert

suggested that blood progesterone concentrations may not have been maintained in all cattle throughout the 7-d protocol.

In progestin-treated cattle, the development of persistent follicles can be avoided by the administration of exogenous steroids at the initiation of progestin treatment. However, administration of estradiol to cattle with low endogenous progesterone concentrations had variable effects on follicle development (Bó et al., 1994; Burke et al., 1997b). In cattle with luteal phase progesterone concentrations, estradiol treatment reduced both plasma FSH concentrations (Martínez et al., 2003a) and LH pulse frequency (Price and Webb, 1988; Diskin et al., 2002), resulting in follicle regression, followed by synchronous follicular wave emergence (Bó et al., 1994). We hypothesized that the ability of estradiol to consistently induce follicle regression (and synchronize follicular wave emergence) at any stage of the estrous cycle is dependent on elevated plasma progesterone concentrations. Since a proportion of cattle are expected to have low plasma progesterone concentrations when a CIDR-based synchronization protocol was initiated at random stages of the estrous cycle, progesterone has been included with the estradiol treatment, even when a new CIDR insert was used. Nevertheless, in the present study, fertility was not improved by the addition of an injection of progesterone at the time of insertion of a new or used CIDR in cattle at random stages of the estrous cycle. In this regard, Burke et al. (1998) demonstrated that progesterone from a new CIDR insert was sufficient to facilitate estradiol-induced follicle atresia in heifers in which luteolysis had been induced with an injection of PGF. This also suggests that fertility would not be compromised when treatment with estradiol and a new CIDR insert is initiated in cattle

with low progesterone concentrations. However, other studies suggest that a combination of estradiol and progesterone did not consistently induce follicular atresia in cattle with low concentrations of circulating progesterone e.g., late in the estrous cycle (Martínez et al., 2002a; Colazo et al., 2003b, Chapter 5). Further studies are needed to characterize the effect of estradiol and progesterone treatments on follicular dynamics and fertility in cattle treated late in the estrous cycle.

In the second experiment, fertility differed between the two locations; cattle at Location A had a lower pregnancy rate than those at Location B. One possible reason of this finding may have been the shorter postpartum interval in cows at Location A; the number of estrous periods prior to insemination has been shown to affect conception rate in dairy cows (Thatcher and Wilcox, 1973; Darwash et al., 1997). Although the same probably applies to beef cattle, low pregnancy rates in early post-partum beef cows have also been associated with development of a CL with a short life-span (Manns et al., 1983). In the present study, 97.1% of the cows were cycling (based on the presence of luteal tissue) at the beginning of the experiment. Furthermore, progestin treatment prior to the induction of ovulation in post-partum cows has been shown to result in a CL with a normal life span (Ramirez-Godinez et al., 1981). However, fertility may have been adversely affected by ovulation of a low quality oocyte, embryo death, or an oviductal or uterine environment hostile to the developing embryo (Butcher et al., 1992; Inskeep, 1995).

Cows (in particular Location A) had a high CIDR loss rate, which seemed higher than that reported by others (Macmillan and Peterson, 1993; Lucy et al., 2001), but was similar to that reported by Martínez et al. (2000a). In Experiment 1, the CIDR loss rate tended to be higher in heifers receiving a used CIDR than in those receiving a new CIDR. Since the nylon filament attached to the CIDR was trimmed, it is unlikely that the loss rate was due to pen-mates pulling the CIDR out. In Experiment 2, cows that lost their device had a significantly shorter postpartum interval than cows that did not; the tonicity of the vaginal wall may increase with postpartum interval and a used CIDR may not press as tightly against the vaginal wall as a new CIDR.

Although pregnancy rates were low in cows that lost their CIDR in this study, Martínez et al. (2000a) did not detect differences in estrus or pregnancy rates between cows that lost CIDR inserts and those that did not. However, in the present study, a number of cows (n = 7) that lost CIDRs were observed in estrus prior to the second EB treatment; these cows were unlikely to become pregnant following TAI. Burke et al. (2000) observed that the administration of 1 mg EB during diestrus shortened luteal lifespan, suggesting that the administration of estradiol in this study may have induced luteolysis, at least in some cattle. Therefore, some cows that lost their CIDRs may have had premature luteal regression, and as a result, ovulated prior to TAI. In any case, the loss of the used CIDRs in this study was associated with a reduced pregnancy rate, which would have diminished or even nullified the economic benefits of CIDR re-use.

Although there are apparently no reports of disease transmission associated with the re-use of CIDR inserts, there is considerable potential for this to occur. In previous experiments (Colazo et al., 2003b, Chapter 5) we have soaked used CIDRs in a povidone-iodine-based detergent, followed by scrubbing with a brush and rinsing with water to remove detergent and debris. The efficacy of this approach for prevention of disease is unknown. Although prolonged exposure to detergents may decrease the potential for disease transmission, it may also contribute to increased losses of progesterone. In the current experiments, CIDR inserts were autoclaved to minimize the risk of disease transmission. There are apparently no reports of plasma progesterone concentrations in cattle that received a washed as compared to an autoclaved CIDR. A further consideration for CIDR re-use is that the insert should be completely dry prior to prolonged storage, to minimize potential for microbial growth.

In summary, pregnancy rates following TAI did not differ between cattle synchronized with a new- or once-used CIDR. However, cattle treated with a twice-used CIDR had a lower pregnancy rate, and the inclusion of two twice-used CIDRs did not significantly improve pregnancy rates following TAI. The addition of 100 mg progesterone to estradiol treatment (to synchronize follicular wave emergence) did not affect pregnancy rates, regardless of whether a new or used CIDR insert was used. Pregnancy rates following TAI were affected by parity; heifers were more fertile than cows. Overall, pregnancy rates following TAI were acceptable whether a new or a once used-CIDR was used and whether or not progesterone was administered at the time of CIDR insertion.

9.0 RESYNCHRONIZATION OF PREVIOUSLY TIMED-INSEMINATED BEEF HEIFERS WITH PROGESTINS

9.1 Abstract

The objective was to determine the efficacy of a previously used CIDR insert or melengestrol acetate (MGA; 0.5 mg/head/d) for resynchronization of estrus in beef heifers not pregnant to timed-AI (TAI). In three experiments and a field trial, heifers were re-inseminated 6 to 12 h after first detection of estrus. Pregnancy diagnosis was done approximately 25 to 43 d after either TAI or the second AI. In Experiment 1, 79 heifers received a once-used CIDR from 13 to 20 d after TAI and 80 heifers were untreated controls. For these two groups, there were 34 and 35 heifers, respectively, not pregnant following TAI; mean ± SD intervals from TAI to onset of estrus were 21.9 ± 1.1 d versus. 19.0 ± 2.1 d (means, P<0.001; variance, P=0.07); estrus rates were 70.6 versus. 85.7% (P=0.1); conception rates were 62.5 versus. 76.7% (P<0.3); and pregnancy rates were 44.1 versus. 65.7% (P=0.07). In Experiment 2, heifers (n=651) were TAI (Day 0) and 13 d later randomly assigned to one of seven groups (n=93 per group) to receive a once-used CIDR (three groups; Days 13 to 20), MGA (three groups; Days 13 to 19), or no treatment (Control). The three groups were: no further treatment (CIDR or MGA alone), 1.5 mg estradiol-17β (E-17β) and 50 mg progesterone (P) in 2 mL canola oil on Day 13, or E-17β and P on Day 13 and 0.5 mg E-17β on Day 21 (24 h after CIDR removal or 48 h

after the last feeding of MGA). Pregnancy rate following TAI was lowest (P<0.05) for the group given a CIDR and E-17β and P on Day 13 and E-17β on Day 21. Variability in return to estrus was greater (P<0.001) in the control than in progestin-treated groups. Conception and pregnancy rates in heifers given a CIDR (65.1 and 61.4%) were higher (P<0.01) than those fed MGA (49.6 and 40.4%), but not different from controls (62.2 and 54.9%, respectively). In Experiment 3, 616 heifers received a once- or twice-used CIDR for 7 d, beginning 13 ± 1 d after TAI, with or without a concurrent injection of 150 mg of P, in a 2 x 2 factorial design. Pregnancy rate following TAI was 47.2%. In heifers that returned to estrus, there was no significant difference between once- or twice-used CIDR for rates of estrus (68.8%, P<0.3), conception (65.9%, P<0.6) and pregnancy (45.3%, P<0.8). Injecting progesterone at CIDR insertion increased the interval from CIDR removal to onset of estrus (2.5 versus. 2.3 d, P<0.05) and reduced rates of estrus (63.8 versus. 73.8%, P<0.05), conception (60.5 versus. 70.6%, P=0.1) and pregnancy (38.6 versus. 52.2%, P<0.02). In a field trial, 983 heifers received a once-used CIDR for 7 d, beginning 13 ± 1 d after TAI. Pregnancy rate following TAI was 55.2%. The median (and mode) of the interval from CIDR removal to estrus was 2.5 d. Estrus, conception and pregnancy rates were 78.2, 70.3 and 55.0% (overall pregnancy rate following TAI and rebreeding, 78.7%). In summary, a once- or twice-used CIDR for 7 d, starting 13 ± 1 d after TAI resulted in the majority of nonpregnant heifers detected in estrus over a 4-d interval, with acceptable conception rates; however, injecting progesterone at CIDR insertion significantly reduced both estrus and pregnancy rates, and estradiol treatment after CIDR removal decreased pregnancy rate following TAI. Fertility was higher in heifers resynchronized with a once-used CIDR than with MGA.

9.2 Introduction

There are several progestin-based protocols for synchronizing ovulation in cattle, enabling timed-artificial insemination (TAI) (Martínez et al., 2000a; 2002b; Colazo et al., 2003b, Chapter 5; Colazo et al., 2004a, Chapter 6). Most cattle that fail to become pregnant following TAI return to estrus 15 to 25 d later (Van Cleef et al., 1996; Chenault et al. 2003). Hence, intensive estrus detection and re-insemination of cattle returning to estrus is required to maximize the number of cattle becoming pregnant to AI. Although the duration of estrus detection and rebreeding could be minimized by either inserting a previously used CIDR insert (Macmillan and Peterson, 1993; Van Cleef et al., 1996; Stevenson et al., 2003) or feeding MGA (Stevenson et al., 2003), fertility may be compromised by prolonged growth and dominance of the ovulatory follicle in cattle with luteal regression prior to progestin withdrawal (Stevenson et al., 2003). Therefore, in the absence of treatments to induce a new follicular wave, fertility to a re-insemination in progestin-treated cattle could be compromised by ovulation of a persistent follicle (Savio et al., 1993).

Since pregnancy diagnosis by transrectal ultrasonography is usually not performed until at least 27 to 28 d after AI, treatments designed to induce a new follicular wave should not jeopardize pregnancy rate following TAI. Although estradiol benzoate treatment increased both the percentage of non-pregnant cows returning to estrus (Macmillan et al., 1997; Stevenson et al., 2003) and conception rates at the resynchronized estrus (Macmillan et al., 1997), estradiol benzoate administered on Day

13 after TAI also decreased pregnancy rate in beef heifers (Cutaia et al., 2002). The use of a shorter-acting estradiol (i.e. estradiol-17β) warrants investigation. Furthermore, since progesterone treatment (100 or 200 mg per se, or a CIDR insert for at least 24 h) induced atresia of persistent follicles (Anderson and Day, 1994; Cavalieri et al., 1998), we hypothesized that progesterone could be used to synchronize ovarian follicular development and returns to estrus in nonpregnant heifers without adverse effects on pregnant heifers.

The objectives of the present studies were to evaluate the efficacy of a used CIDR insert (once- or twice-used) or MGA feeding for resynchronization of estrus in beef heifers not pregnant following TAI, and to determine whether an injection of estradiol-17β (E-17β) or a commercial progesterone preparation could be used to increase estrus and pregnancy rates in these heifers.

9.3 Materials and Methods

9.3.1 Experiment 1

The objective was to compare the efficacy of a used CIDR with no treatment on estrous activity, conception rate, and pregnancy rate in heifers nonpregnant following TAI. This study was replicated at two locations.

In Replicate 1, 99 Hereford and Hereford-cross heifers (approximately 13 to 14 mo of age, 350 to 450 kg) were used. The heifers were maintained in a feedlot and were fed a silage-based ration, supplemented with rolled barley. Drinking water, trace-mineralized salt and a calcium-phosphorous mineral supplement were available ad libitum. Heifers were randomly assigned to two synchronization protocols for TAI (Day of TAI designated as Day 0; Kastelic et al., 2001).

In Replicate 2, 60 Angus and Angus-cross heifers (similar age, size, weight, housing, and feeding as in Replicate 1) were used. The experimental design was identical to that of Replicate 1, with the difference that just prior to initiating the TAI protocol, all heifers were examined by transrectal ultrasonography (Aloka 500 with 7.5 MHz linear-array transrectal transducer; ISM Inc., Edmonton, AB, Canada); only heifers with a corpus luteum detected by ultrasonography were used in this replicate.

On Day 13 after TAI, heifers were allocated (by replicate, within the previous treatment groups) to receive either a once-used intravaginal controlled internal drug release insert (CIDR; Bioniche Animal Health Inc., Belleville, ON, Canada) for 7 d, or no CIDR insert (Controls). Before reuse, CIDR inserts were soaked in water containing povidone iodine (Betadine Scrub; Purdue Pharma, Pickering, ON, Canada) for approximately 2 h, individually scrubbed with a brush, thoroughly rinsed, and allowed to completely air-dry.

In Replicate 1, all heifers were fitted with a pressure-sensing mount detector (HeatWatch^{TM}; DDx Inc., Boulder, CO, USA) on Day 13 (at CIDR insertion in the treated group) and were monitored continuously for mounting activity until Day 26. Heifers detected in estrus (defined as ≥ 4 mounts for at least 2 sec in duration within a 6-h interval) were reinseminated with commercial frozen-thawed semen approximately 9 to 12 h after the first mount. In Replicate 2, visual observation for estrus detection was performed for periods of 40 min three times daily from Days 13 to 26; heifers were reinseminated 6 to 12 h after first detection of standing estrus.

Transrectal ultrasonography for pregnancy diagnosis was conducted on Day 30 in all heifers that had not been detected in estrus and rebred, and on approximately Day 60 in all rebred heifers. Pregnant, rebred heifers were examined carefully to determine if the stage of pregnancy was consistent with conception to the first or the second insemination.

Estrus, conception and pregnancy rates were defined as follows:

Estrus rate = [(number of heifers detected in estrus)/(number of heifers detected in estrus + number of heifers diagnosed nonpregnant 30 d after TAI)] x 100.

Conception rate = [(number of heifers pregnant to rebreeding)/(number of heifers rebred)] x 100.

Pregnancy rate = [(number of heifers pregnant to rebreeding)/(number of heifers detected in estrus + number of heifers diagnosed nonpregnant 30 d after TAI)] x 100.

9.3.2 Experiment 2

Angus and Angus cross heifers (n=651), 12 to 15 mo of age and weighing approximately 300 to 375 kg, were used between April and June, 2000. Heifers were fed barley silage and had ad libitum access to water, trace-mineralized salt, and a calcium-phosphorous mineral supplement. Heifers underwent TAI (designated as Day 0) and 13 d later were randomly assigned to seven groups (n=93 per group) to receive a once-used CIDR (three groups) for 7 d (Days 13 to 20), melengestrol acetate (MGA; Pharmacia Animal Health, Orangeville, ON, Canada; 0.5 mg/head/day; three groups) for 7 d (Days 13 to 19), or no treatment (Control). The three groups were: no further treatment (CIDR or MGA alone), 1.5 mg estradiol-17β (E-17β) and 50 mg progesterone (P; both from Sigma Chemical Co, St. Louis, MO, USA) in 2 mL canola oil on Day 13, or E-17β and P on Day 13 and 0.5 mg E-17β on Day 21 (24 h after CIDR removal or 48 h after the last feeding of MGA). Estrus detection was done by visual observation (as in Replicate 2, Experiment 1) from Days 13 to 30. Heifers detected in estrus were re-inseminated 6 to 12 h after onset of estrus by one technician, using commercial frozen-thawed semen from one of two bulls (the bulls were balanced across treatment groups). Pregnancy diagnosis by ultrasonography was performed approximately 43 d after rebreeding.

9.3.3 Experiment 3

The objective was to investigate the efficacy of a once- or twice-used CIDR insert, with or without supplemental progesterone, for resynchronization of estrus in beef heifers not pregnant following TAI. Angus and Angus-cross beef heifers (n = 616), 12 to 15 mo of age and 280 to 400 kg of weight were housed in a drylot and fed a barley-silage based diet. All heifers had been inseminated by TAI (Colazo et al., 2004b, Chapter 8) and were assigned randomly to four groups (2 x 2 factorial) 13 ± 1 d later to receive a once- or twice-used CIDR for 7 d, with or without a concurrent injection of 150 mg im of progesterone (Progesterone 5%; Vétoquinol N.-A Inc., Lavaltrie, QC, Canada). Estrus detection and inseminations were performed as in Experiment 2. Ultrasonographic pregnancy diagnosis was done approximately 30 d after rebreeding.

9.3.4 Field trial

This was a large-scale trial of previously-used CIDR for resynchronization of returns to estrus following TAI. The study was replicated simultaneously at two feedlots, approximately 50 km apart. Angus and Angus-cross heifers, 12 to 15 mo of age and weighing approximately 325 to 375 kg were used. Heifers were fed a base ration of barley silage at both locations, supplemented with approximately 10% of either barley grain or malt-sprout pellets at the two locations, respectively. Heifers had ad libitum access to clean drinking water, cobalt iodized salt, and a calcium-phosphorous mineral supplement.

Just prior to the start of the synchronization protocols for TAI, heifers were examined by transrectal ultrasonography. Freemartin heifers and those without an ultrasonically detectable luteal structure and (or) with ovarian follicles <10 mm in diameter and a uterine diameter <15 mm were excluded. A total of 983 heifers were assigned to three different estrus synchronization protocols (Colazo et al., 2004a, Chapter 6) and TAI was done on three consecutive days. On Day 13 ± 1 after TAI, all heifers received a previously-used CIDR for 7 d. Following CIDR removal, estrus detection and re-inseminations were done for 5 d. Ultrasonographic pregnancy diagnosis was conducted as described in Experiment 1.

9.3.5 Statistical Analyses

In Experiments 1 and 2, associations between pregnancy status following TAI, estrus, conception and pregnancy rates to rebreeding and treatment groups were analyzed using generalized estimating equations (GEE; PROC GENMOD in SAS). Model specifications included a binomial distribution, logit link function, repeated statement with subject equal to pen, and an exchangeable correlation structure. The model consisted of replicate (Experiment 1), treatments and their interactions. Treatments were analyzed as a fixed effect with two (Experiment 1) or three (Experiment 2) categories.

Detailed analysis of mounting data in Experiment 1 was confined to Replicate 1. A Student's t-test was used to compare the effects of a used CIDR on the interval from TAI to onset of estrus, duration of estrus, and number of mounts. Bartlett's test of homogeneity of variance was used to compare, between the two groups, the variance of

the interval from TAI to the onset of estrus. One-way ANOVA compared the intervals from TAI to rebreeding among treatment groups in Experiment 2. In Experiment 3, the main effects of CIDR (once- versus. twice-used), progesterone treatment, and their interaction, on interval from CIDR removal to estrus was determined by two-way ANOVA. Means were compared with a protected LSD test. Comparisons of equality of variances among groups were done by Bartlett's test of homogeneity of variance. The Mantel-Haenszel test (Chi-square test for a 2x2 experimental design) was used to detect differences among treatment groups for estrus rates, conceptions and pregnancy rates following TAI and to rebreeding. Pregnancy rate between treatments by day was analyzed with Fisher's exact test. As the field trial was demonstrational, no statistical analysis was done. All statistical analyses, except the Mantel-Haenszel test were conducted with the Statistical Analysis System (SAS; Version 8.2 for Windows; SAS Institute, Cary, North Carolina, USA).

9.4 Results

9.4.1 Experiment 1

CIDR treatment did not affect pregnancy rate following TAI (57.0 versus 56.4% for CIDR and control groups, respectively; $P<0.7$). There were no significant differences between the two replicates for estrus, conception or pregnancy rates ($P=0.3$). Combined for both replicates, there were 69 heifers not pregnant to the first AI; 34 heifers received a used CIDR, while 35 heifers were untreated controls. The proportions of nonpregnant

heifers detected in estrus and rebred were 24/34 (70.6%) versus. 30/35 (85.7%; P=0.13), and conception and pregnancy rates were 62.5 versus. 76.7% (P=0.3) and 44.1 versus. 65.7% (P=0.07) for CIDR-treated and control groups, respectively.

Mounting activity associated with rebreeding was characterized, in detail, in Replicate 1. Mean (± SD) intervals from TAI to onset of estrus were 21.9 ± 1.1 d versus. 19.0 ± 2.1 d (means, P<0.001; variance, P=0.07) for CIDR-treated and control groups, respectively. However, there were no differences between groups for duration of estrus (overall, 10.5 ± 7.2 h; P=0.3) or number of mounts (overall, 18.8 ± 15.3; P=0.3). Distribution of estrus and the number of heifers that became pregnant are shown in Figure 9.1.

It was noteworthy that there were two heifers in Replicate 1 that were detected in estrus and re-inseminated, but subsequently diagnosed pregnant following TAI. Both of these heifers had received a used CIDR. The first heifer was mounted nine times over a 7-h interval, 20 d after TAI. The second heifer was mounted four times over a 2-h interval 22 d after TAI, and an additional four times over a 45-min interval, approximately 24 h later.

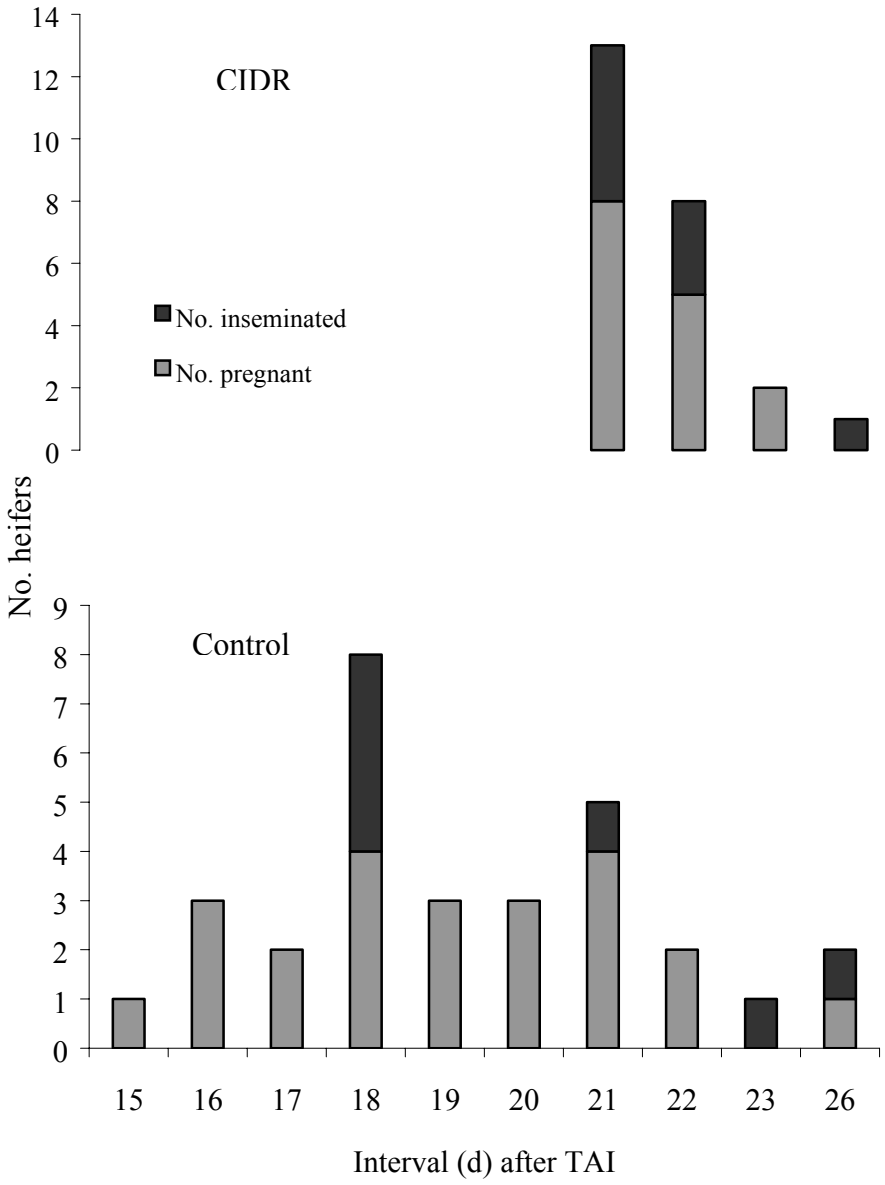

Figure 9.1. Number of heifers re-inseminated and pregnant, grouped by interval following timed-AI (TAI). Heifers received a once-used CIDR insert for 7 d beginning 13 ± 1 d after TAI (CIDR; n = 34) or no treatment (Control; n = 35). Estrus rate tended to be higher (85.7 versus. 70.6%; P=0.1), but more variable (P=0.07) in control than in CIDR-treated heifers. Pregnancy rate also tended (P=0.07) to be higher in control than in CIDR-treated heifers.

9.4.2 Experiment 2

Reproductive outcome by group is shown in Table 9.1. Variability in the interval to estrus was greater in the control than in progestin-treated groups ($P<0.001$; Figure 9.2). The median interval to estrus was 2.5 d after CIDR removal and 3.5 d after the last feeding of MGA. Conception and pregnancy rates in heifers diagnosed not pregnant 30 d after TAI and given a CIDR (65.1 and 61.4%) were greater ($P<0.01$) than those fed MGA (49.6 and 40.4%), but were not different from controls (62.2 and 54.9%, respectively). Pregnancy rate following TAI was lower ($P<0.05$) for the group given a used CIDR, E-17β and P on Day 13 and E-17β on Day 21, and tended ($P=0.09$) to be lower in the control group and heifers fed MGA and receiving E-17β and P on Day 13 than in the other groups (Table 9.1).

Figure 9.2. Number of previously TAI heifers seen in estrus and re-inseminated, grouped according to treatment (CIDR, Fig. A; MGA, Fig. B, and control Fig. C). Heifers (n= 651) were allocated to receive a once-used CIDR for 7 d (C), C plus 1.5 mg of E-17β and 50 mg P on Day 13 (CEP), CEP plus 0.5 mg of E-17β on Day 21 (CEPE), MGA (0.5 mg/head/d) for 7 d (M), M plus 1.5 mg of E-17β and 50 mg P on Day 13 (MEP), MEP plus 0.5 mg of E-17β on Day 21 (MEPE), or no treatment (Control). Variability in the interval to estrus was greater in the control than in progestin-treated groups ($P<0.001$).

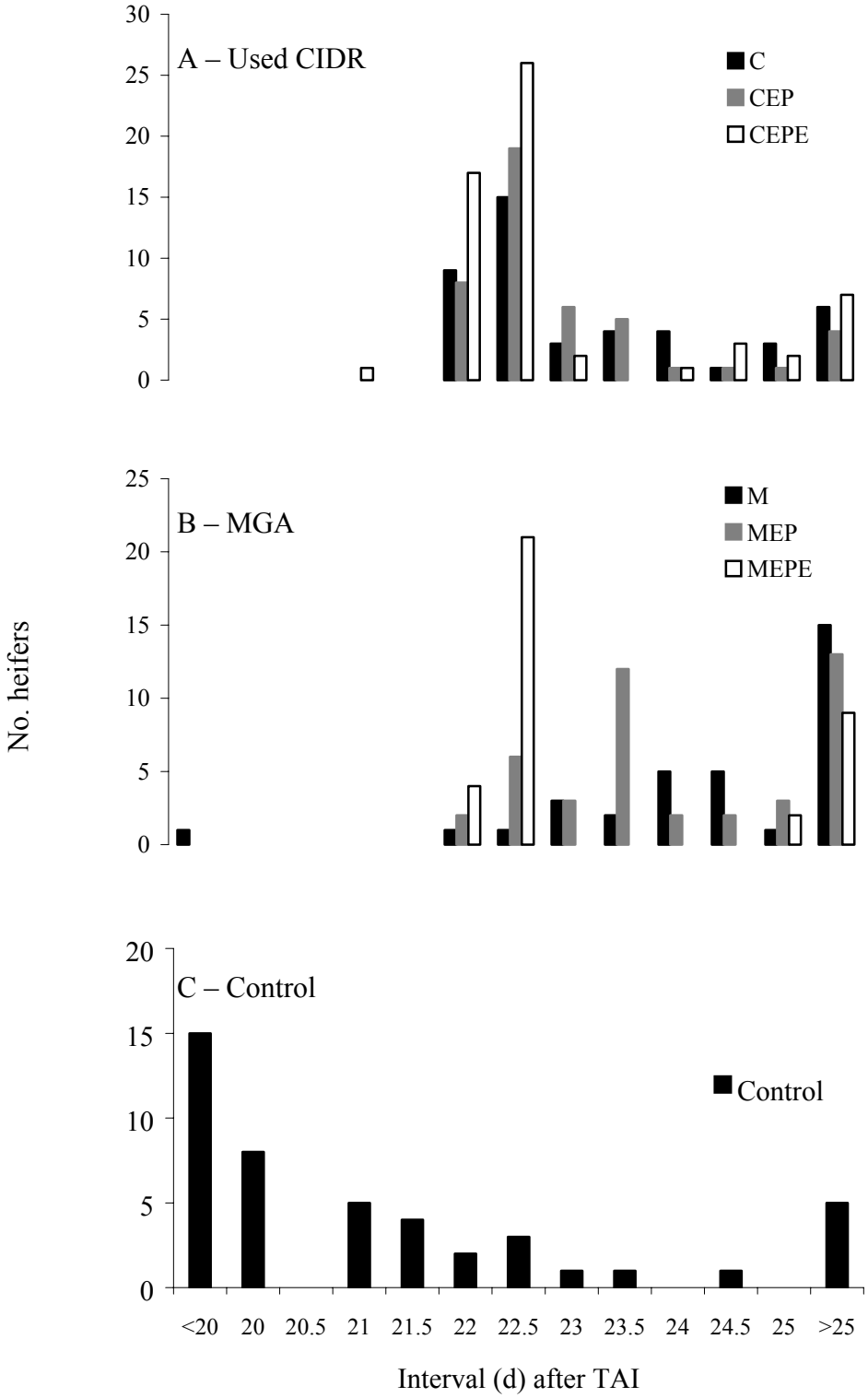
A – Used CIDR
C
CEP
CEPE
B – MGA
M
MEP
MEPE
C – Control
Control
No. heifers
<20
20
20.5
21
21.5
22
22.5
23
23.5
24
24.5
25
>25
Interval (d) after TAI

Table 9.1. Reproductive outcome of resynchronized heifers starting 13 d after TAI (TAI=Day 0). Heifers (n= 651) were allocated to receive a once-used CIDR for 7 d (C), C plus 1.5 mg of E-17β and 50 mg P on Day 13 (CEP), CEP plus 0.5 mg of E-17β on Day 21 (CEPE), MGA (0.5 mg/head/d) for 7 d (M), M plus 1.5 mg of E-17β and 50 mg P on Day 13 (MEP), MEP plus 0.5 mg of E-17β on Day 21 (MEPE), or no treatment (Control).

	C	CEP	CEPE	M	MEP	MEPE	Control
No. heifers	93	93	93	93	93	93	93
No. pregnant following TAI	44	46	31	51	42	45	42
Pregnancy rate (%)[1]	47.3[a]	49.5[a]	33.3[b]	54.8[a]	45.2[ab]	48.4[a]	45.2[ab]
Rebreeding							
No. open following TAI	49	47	62	42	51	48	51
No. in estrus	45	45	59	35	43	37	45
Estrus rate (%)[2]	91.8[ab]	95.7[a]	95.2[a]	83.3[bc]	84.3[bc]	77.1[c]	88.2[ac]
Rebred mean (Day)	23.5[a]	23.1[ab]	23.1[bc]	24.9[bc]	24.3[c]	23.7[c]	20.8[d]
Variance (d)	3.5[xy]	2.3[x]	3.3[xy]	4.4[y]	2.8[x]	4.6[y]	10[z]
Range	22-29.5	22-29	21-28.5	19-29.5	22-29	22-29	14-28
No. pregnant	29	33	35	14	23	20	28
Conception rate (%)	64.4[ab]	73.3[a]	66.1[ab]	40[c]	53.5[bc]	54[bc]	62.2[ab]
Pregnancy rate (%)[3]	59.2[ab]	70.2[a]	56.4[ab]	33.3[c]	45.1[bc]	41.7[bc]	54.9[ab]
TAI & rebreeding							
Pregnancy rate (%)[4]	78.5[ab]	84.9[a]	71[b]	69.9[b]	69.9[b]	69.9[b]	75.3[ab]

[abc] Proportions within a row differed (P<0.05).
[abcd] Means within a row differed (P<0.0001).
[xyz] Variances within a row differed (P<0.05)
[1] CEPE versus MEP and control (P=0.09).
[2] Control versus MEPE (P=0.1).
[3] C versus MEPE (P=0.08), CEP versus control and CEPE versus MEPE (P=0.1).
[4] CEP versus control (P=0.09).

9.4.3 Experiment 3

Overall pregnancy rate following TAI was 47.2% (291/616; Colazo et al., 2004b, Chapter 8). Although 325 heifers were diagnosed nonpregnant following TAI, five of these heifers (1.5%) had no CIDR present at the time of scheduled removal (20 ± 1 d after TAI); therefore, data from only 320 resynchronized heifers were used for analysis (Table 9.2). Resynchronization treatments did not adversely affect pregnancy rates following TAI (P=0.8 and 0.7 for CIDR and progesterone-treated animals, respectively). The interval from CIDR removal to the onset of estrus was not affected by the number of times that the CIDR had been previously used (overall, 2.4 ± 0.5 d; P=0.85). The mean (± SD) interval from CIDR removal to estrus tended (P=0.06) to be longer in heifers given an injection of progesterone concurrent with CIDR insertion (2.5 ± 0.6 d) as compared to those that did not receive this treatment (2.3 ± 0.5 d). Pregnancy rate tended (P=0.1) to be higher in untreated heifers reinseminated 1.5 and 2 d after CIDR removal than in progesterone-treated heifers.

Table 9.2. Estrus, conception, and pregnancy rates in beef heifers* receiving a once- or twice-used CIDR with (P) or without (no P) an injection of 150 mg im of a commercial progesterone preparation 13 ± 1 d after timed-AI.

			Resynchronization		
	CIDR	Pregnancy rate following TAI	Estrus rate	Conception rate	Pregnancy rate
No P	Once-used (%)	43.3	79.2	73.7	58.3
	n	55/127	57/72	42/57	42/72
	Twice-used	50.0	69.4	67.8	47.1
		87/174	58/85	40/59	40/85
	Total	47.2	73.8[a]	70.6[c]	52.2[a]
		142/301	116/157	82/116	82/157
P	Once-used	51.5	65.0	53.8	35.0
		86/167	52/80	28/52	28/80
	Twice-used	42.6	62.6	67.3	42.2
		63/148	52/83	35/52	35/83
	Total	47.3	63.8[b]	60.5[d]	38.6[b]
		149/315	104/163	63/104	63/163
Overall		47.2	68.7	65.9	45.3
		291/616	220/320	145/220	145/320

* Heifers diagnosed nonpregnant 28 d after TAI
[ab] Within a column, proportions with different superscripts were different (P<0.05)
[cd] Within a column, proportions with different superscripts tended to differ (P=0.1)

9.4.4 Field trial

Mounting activity was noted approximately 4 d after TAI in 19 heifers; all were re-inseminated, considered nonpregnant following TAI, and excluded from resynchronization data. Pregnancy rate following TAI was 55.2% (542/983). Estrus detection and AI were done on 336 heifers over a 4-d interval (between 1.5 and 5.5 d after CIDR removal; Figure 9.3). The mean (± SD) interval from CIDR removal to AI was 2.6 ± 0.8 d. Six heifers (1.1%) detected in estrus and re-inseminated were subsequently diagnosed pregnant following TAI. Overall, estrus, conception, and pregnancy rates following resynchronization were 78.2% (330/422), 70.3% (232/330), and 55.0% (232/422), respectively. The overall pregnancy rate following TAI plus re-insemination was 78.7% (774/983).

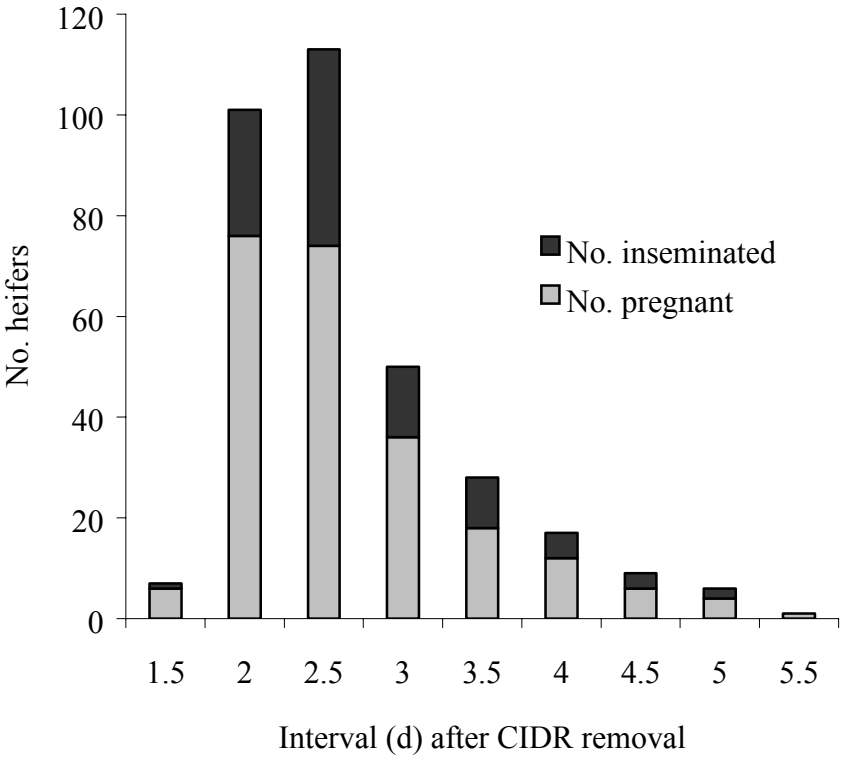

Figure 9.3. Number of heifers reinseminated and pregnant in the field trial, grouped according to interval to onset of estrus after CIDR removal (Day 0). A once-used CIDR was inserted in beef heifers (n = 983) 13 ± 1 d after timed-AI and subsequently removed 7 d later. Estrus was detected by visual observation for 5 d and re-inseminations were done 6 to 12 h after first detection of estrus.

9.5 Discussion

The rationale for insertion of a previously used CIDR, or of feeding MGA, was to delay estrus in heifers that were not pregnant following TAI and may have had early luteolysis. Progestin treatment (starting 13 ± 1 d after TAI) synchronized returns to estrus; compared to control heifers, the range was reduced by 6 and 9 d in Experiments 1 and 2, respectively. The numbers of heifers showing behavioural estrus peaked approximately 21-22.5 d after TAI in progestin-treated heifers; most of the control heifers were rebred before Day 20 after TAI. Overall, estrus rates ranged from 68.8 to 95.7% in progestin-treated heifers and from 85.7 to 88.2% in control heifers

For unknown reasons, the effects of progestins on estrus rate varied between Experiments 1 and 2. In that regard, estrus rate tended to be lower in CIDR-treated than control heifers in Experiment 1, whereas in Experiment 2, estrus rates in CIDR-treated and MGA-treated heifers were not significantly different from that of control heifers. Overall, the timing of estrus and estrus rates were consistent with previous reports. Van Cleef et al. (1996) observed only 80% of nonpregnant heifers in estrus between 12 and 45 d after AI. In that study, reinsertion of a used CIDR from 17 to 22 d after AI resulted in approximately 60% of the non-pregnant heifers detected in estrus over 4 d, compared to 17% of control heifers over the same interval (Van Cleef et al., 1996).

In Experiment 1, the conception rate in CIDR-treated heifers was lower than in control heifers (62.5 versus 76.7%), but the difference was not significant (unfortunately this study had limited power to detect a difference). Since fertility could be compromised by the development of persistent follicles in cattle destined to return to estrus before CIDR removal (Savio et al., 1993), treatments to reset the follicle wave were administered in Experiments 2 and 3. However, treatments designed to induce a new ovarian follicular wave must not induce luteolysis in cattle with unknown pregnancy status. Although estrogens are highly efficacious in synchronizing ovarian follicle development, it is well established that endogenous estrogens from the preovulatory follicle trigger the cascade that induces luteolysis (Niswender et al., 2000), and that exogenous estrogens can induce luteolysis (Wiltbank et al., 1961). The efficacy of estrogens to induce luteolysis appears to depend on both the formulation (i.e. ester) and dose. Treatment with 5 mg of estradiol valerate induced luteolysis, but lower doses were ineffective (Wiltbank et al., 1961; Colazo et al., 2005, Chapter 7). Similarly, plasma progesterone concentrations decreased from 2 to 7 d after treatment with 5 mg of estradiol benzoate (Munro and Moore, 1985), while 200 μg given twice daily for 3 d had no effect on mean cycle length (Hixon et al., 1983). It was noteworthy that 1 mg of estradiol benzoate given for resynchronization of returns to estrus compromised the ability of the CL to produce progesterone for at least 48 h in dairy cows (Burke et al., 2000), and significantly decreased pregnancy rates following TAI in beef heifers (Cutaia et al., 2002). Estradiol-17β (E-17β; the active metabolite of estradiol esters) has also been used to synchronize ovarian follicular wave emergence and ovulation in cattle. Peak plasma estradiol concentrations occurred sooner and were higher in cattle treated with E-

17β than those treated with estradiol valerate or benzoate (Bó et al., 1993; 2000; Martínez et al., 2003a). Furthermore, there are no reports that low doses of E-17β will cause luteolysis.

Recently, Bó et al. (2000) demonstrated that 1 mg of E-17β was as effective as 5 mg of E-17β in suppressing follicles in progestogen-implanted heifers, with emergence of a new follicular wave 3 to 5 d later. Therefore, E-17β was used to induce a new follicular wave and estrus in Experiment 2. In this study, the administration of 1.5 mg of E-17β and 50 mg of P at CIDR insertion did not adversely affect pregnancy rate following TAI, but the administration of 0.5 mg E-17β 24 h after CIDR removal was associated with a reduction in pregnancy rate following TAI. Alternatively, treatment of MGA-fed heifers with a 1.5 mg E-17β and 50 mg P at the beginning of the feeding period tended to result in reduced pregnancy rates following TAI, while the same treatment followed by 0.5 mg E-17β 48 h after the last feed of MGA did not. Collectively, these data are difficult to rationalize. Although they could be a result of chance, luteolysis may have occurred in E-17β-treated heifers, or the administration of E-17β after CIDR removal may have resulted in the re-insemination of pregnant heifers.

Failure to detect estrus in heifers that were subsequently diagnosed nonpregnant could have been due to unobserved estrus or embryonic loss (and returning to estrus after estrus detection had ceased). It is recognized that cattle without a viable embryo on Day 16 return to estrus within 24 d after AI, whereas embryo loss after Day 16 delays returns to estrus (Betteridge et al., 1980; Humblot, 2001). We attempted to increase the

proportion of nonpregnant heifers detected in estrus by giving estradiol after progestin withdrawal in Experiment 2. However, 90 of 96 nonpregnant heifers receiving a used CIDR and no estradiol treatment after CIDR removal were detected in estrus, making it very difficult to show a benefit for the use of estradiol. Estrus rates were also relatively high (approximately 84%) in MGA-treated heifers and there was no benefit of estradiol treatment after the termination of MGA. The effect of adding estradiol to progestin-based resynchronization protocols on the proportion of cattle returning to estrus after TAI has been controversial. In previous studies, treatment with estradiol esters (benzoate or cypionate) at CIDR insertion and 24 h after CIDR withdrawal increased (Macmillan et al., 1997; Stevenson et al., 2003) or did not increase (El-Zarkouny and Stevenson, 2004) the percentage of non-pregnant cows returning to estrus. In another study, the number of beef heifers returning to estrus after TAI was not increased by treatment with 0.5 mg of estradiol cypionate at first feeding and 24 h after last feeding of MGA (Stevenson et al., 2003). Results from CIDR- and MGA-treated heifers in the present study would also indicate no benefit to giving estradiol after cessation of progestin treatment. Regardless, the use of E-17β and progesterone at CIDR insertion and E-17β after CIDR removal can not be recommended for resynchronization of returns to estrus at this time.

There is considerable evidence that blood progesterone concentrations are positively correlated with embryo development and the production of INF-τ (Garret et al., 1988; Mann et al., 1999). However, previous attempts to improve fertility by supplementing progesterone during the luteal phase have yielded inconsistent results (Macmillan and Peterson, 1993; Van Cleef et al., 1996; Chenault et al., 2003). Macmillan

and Peterson (1993) reported that inserting a new CIDR between 4 and 9 d after AI in lactating dairy cows significantly improved pregnancy rates; whereas insertion of a CIDR 10 to 16 d or 14 to 17 d after AI did not. Van Cleeff et al. (1996) also reported that insertion of a used CIDR for 5 d (17 to 22 d after AI) apparently did not affect the proportion of heifers pregnant to the first insemination. Conversely, it has been shown that inserting a new or used CIDR 1 or 2 d after AI in heifers reduced pregnancy rates (Van Cleef et al., 1996). Chenault et al. (2003) also reported a small (4.0%) but significant reduction in pregnancy rate to first breeding in dairy cows that received a new CIDR for 7 d (starting 14 ± 1 d after AI). In Experiment 3, there were no indications that the injection of progesterone at the time of used-CIDR insertion 13 ± 1 d after TAI adversely affected pregnancy rate following TAI. Therefore, further studies involving large numbers of cattle are required to ensure that resynchronization with a used CIDR does not reduce pregnancy rate following TAI.

In the present studies, heifers that received only a used CIDR had conception rates following re-insemination that ranged from 60 to 70%. Macmillan and Peterson (1993) reported conception rates of 65.6% in dairy cows resynchronized with a new CIDR insert. Although Chenault et al. (2003) reported conception rates of 26.7% in dairy cows resynchronized with a new CIDR, this was not statistically different from non-resynchronized cattle. Conversely, Stevenson et al. (2003) reported lower conception rates in heifers receiving a used CIDR to resynchronize returns to estrus (33.3%) as compared to untreated controls (60.0%). It is noteworthy that despite the previously mentioned precautions, treatment with E-17β and progesterone at CIDR insertion on Day

13 in Experiment 2 resulted in nonsignificant increases in rates of estrus, conception and pregnancy, and a small reduction in the variance in returns to estrus (compared to used-CIDR treatment alone). However, the overwhelming benefit for the use a used CIDR to resynchronize returns to estrus would appear to be the reduced amount of time spent on estrus detection. In the field trial, 336 heifers were observed in estrus over a 4-day interval (with a mean interval of 2.6 d from CIDR removal to onset of estrus). Estrus, conception and pregnancy rates were considered highly satisfactory with more than 78% of the heifers pregnant following TAI and re-insemination.

A used CIDR resulted in higher conception and pregnancy rates than the feeding of MGA in nonpregnant heifers resynchronized following TAI (Experiment 2). MGA has been shown to be less effective than progesterone in suppressing serum LH concentrations in cattle without a CL resulting in persistent follicles (Kojima et al., 1995). Consequently, short-term (e.g. 7- or 8-d) MGA-based protocols for TAI typically include treatments such as estradiol, GnRH or LH at the start of MGA feeding to prevent the development of persistent follicles (Savio et al., 1993) These protocols have resulted in pregnancy rates following TAI that have not differed from those of similar protocols utilizing a CIDR (Martínez et al., 2002b). In the present study, giving estradiol and progesterone concurrent with the initiation of MGA feeding (with or without estradiol treatment after the cessation of MGA) resulted in an increase in conception rates that was approximately 14% higher than that achieved with MGA alone; however, conception rates (and pregnancy rates) were still significantly lower than in CIDR-treated heifers given estradiol and progesterone at the time of CIDR insertion. Therefore, MGA would

not appear to be a good alternative for the resynchronization of previously time-inseminated heifers.

In Experiment 3, 150 mg of progesterone was given concurrent with CIDR insertion in an attempt to cause regression of the dominant follicle, and to synchronize follicular wave emergence. Administration of 200 mg of progesterone in *Bos taurus* cattle (Anderson and Day, 1994) or 100 mg progesterone or a new CIDR for 24 h in *Bos indicus* cattle has been reported to induce atresia of persistent follicles (Cavalieri et al., 1998). Although it was not expected that heifers in the present study would have a persistent follicle, the administration of progesterone concurrent with insertion of a used-CIDR resulted in a significantly longer interval from CIDR removal to estrus, suggesting that a new wave of ovarian follicular development may have emerged in response to treatment in at least some heifers. However, progesterone treatment also resulted in reduced rates of behavioural estrus and conception, and as a result, pregnancy rates were also reduced. In particular, progesterone treatment tended to result in reduced fertility in heifers detected in estrus within the first 2 d after CIDR removal. The reason for this observation is not known, but based on the current results, an, injection of supplemental progesterone concurrent with CIDR reinsertion can not recommended. Further studies are needed to investigate alternative approaches to synchronize follicular growth in previously inseminated cattle.

The reuse of CIDR inserts is not recommended by the manufacturer, but there are many reports of successful reuse of CIDR in estrus synchronization programs (Colazo et al., 2004b, Chapter 8). Although the efficacy of cleaning and disinfection procedures utilized in this study is unknown, there are apparently no reports of disease transmission associated with the reuse of CIDR inserts. Our previous studies have indicated a greater potential for CIDR loss with reused CIDR inserts (especially following autoclaving) with reduced pregnancy rates following TAI in those cattle (Colazo et al., 2004b, Chapter 8). However, the loss of used CIDR inserts in this study was extremely low. In addition, twice-used CIDR inserts seemed to perform as well as once-used inserts in the present study. However, fertility was reduced in cattle receiving a twice-used CIDR in a TAI protocol (Colazo et al., 2004b, Chapter 8). Therefore, twice-used CIDR inserts would appear to be efficacious for resynchronization protocols (and perhaps estrus synchronization programs which utilize estrus detection), but they cannot be recommended for TAI protocols.

Detection of estrus followed by AI in pregnant animals when resynchronizing cattle with unknown pregnancy status is of concern; estrus has been reported to occur in 3 to 6% of pregnant cattle (Roberts, 1986). In Experiment 1 and the field trial, two and six heifers, respectively, that were detected in estrus and re-inseminated were subsequently diagnosed pregnant; these heifers represented 4.4 and 1.1% of all heifers pregnant following TAI. However, the number of pregnant heifers that were detected in estrus, rebred, and subsequently lost the TAI pregnancy is not known, and is of greater concern. In previous studies, embryonic or fetal mortality following AI of pregnant cattle has been

estimated to range from 24 to 49% (Sturman et al., 1980; 2000). Based on the low incidence of rebred heifers that were subsequently diagnosed pregnant following TAI and reported rates of embryonic death in re-inseminated cattle, we infer that very few pregnant heifers were detected in estrus and re-inseminated. However, the pregnany rates following TAI in CIDR-treated heifers that received estradiol treatment after CIDR removal is still troublesome. It is not clear whether treatment with E-17β following CIDR removal will cause behavioural estrus in pregnant animals.

In summary, insertion of a once- or twice-used CIDR effectively synchronized returns to estrus in the majority of heifers nonpregnant following TAI; fertility was acceptable, with 70 to 80% of heifers pregnant following TAI and the subsequent re-insemination. However, pregnancy rates were substantially lower when MGA was fed in lieu of using CIDR inserts. Treatment with 1.5 mg of E-17β and 50 mg of progesterone at CIDR insertion was associated with in small increase in conception rate; however, treatment with 0.5 mg of E-17β after CIDR removal was associated with reduced pregnancy rates following TAI. Therefore, giving estradiol to increase the expression of behavioural estrus after cessation of progestin treatment cannot be recommended. Injecting 150 mg of progesterone at CIDR insertion had a detrimental effect on expression of estrus and subsequent pregnancy rates. Future studies will have to be directed toward improving the synchrony of follicle growth and increasing the expression of estrus in cattle not pregnant to an initial insemination without compromising pregnancy rate to the timed-insemination**.**

10.0 OVARIAN FUNCTION, ESTRUS, AND FERTILITY IN BEEF CATTLE RESYNCHRONIZED WITH PROGESTINS AND ECP, GnRH OR PROGESTERONE

10.1 Abstract

Three experiments were conducted to determine the effects of progestins (MGA versus CIDR), and estradiol cypionate (ECP), gonadotrophin releasing hormone (GnRH) or progesterone treatment during diestrus on ovarian follicular dynamics, CL function, estrus and ovulation rates, and fertility in beef cattle. In Experiment 1, nonlactating beef cows received a once-used CIDR insert 12.3 ± 0.7 d after ovulation (Day 0) and were randomly allocated to concurrently receive one of the following four treatments: no further treatment (n=10); 1 mg of ECP (n=11); 100 μg of GnRH (n=11); or 150 mg of progesterone (n=11). On Day 7, all CIDR inserts were removed and half of the cows received 0.5 mg of ECP. Follicular wave emergence was most synchronous ($P<0.0001$), and diameter of the dominant follicle on Day 7 was least variable ($P=0.01$) in cows given GnRH. Intervals from CIDR removal to estrus and to ovulation were not affected by treatment on Day 0 ($P=0.2$). However, 10/43 cows were not seen in estrus following CIDR removal on Day 7 (13.6% were given ECP and 33.3% received no treatment; $P<0.1$). In Experiments 2 and 3, beef heifers were allocated to resynchronization treatments 13 or 14 d after timed-AI (TAI; Day 0) and were given either no treatment or

0.5 mg of ECP on Day 7, and then, reinseminated 6 to 12 h after onset of estrus. In Experiment 2, all heifers (n=675) were fed MGA (0.5 mg/head/d) from Days 0 to 5; on Day 0 they received no further treatment (Control) or 100 μg GnRH. In Experiment 3, heifers (n=317) received a once-used CIDR for 7 d or were fed MGA (as in Experiment 2). Combined for Experiments 2 and 3, pregnancy rate following TAI was 38.1% (treatments did not affect pregnancy rate following TAI). In Experiment 2, estrus rate tended ($P<0.1$) to be higher in heifers given ECP on Day 7 (69.6 versus 62.7%); onset of estrus was more synchronous ($P<0.05$) in heifers given GnRH only or GnRH on Day 0 and ECP at CIDR removal. In Experiment 3, heifers given a CIDR and no further treatment had the most synchronous onset of estrus ($P<0.003$). Furthermore, heifers given a CIDR had higher conception (68.8 versus 56.7%; $P<0.1$) and pregnancy (54.1 versus 39.2%; $P<0.04$) rates than those fed MGA. In summary, GnRH at CIDR insertion in late diestrus synchronized follicular wave emergence without affecting estrous cycle length; 0.5 mg of ECP at CIDR removal tended to increase the number of nonpregnant heifers detected in estrus without affecting fertility following TAI or reinsemination. Fertility was higher in heifers resynchronized with a used CIDR than those fed MGA.

10.2 Introduction

Highly synchronous returns to estrus and distinct estrous behaviour in cattle non-pregnant to timed-artificial insemination (TAI) would facilitate reinsemination. Based on previous resynchronization studies, synchrony of behavioural estrus and the proportion of nonpregnant cattle detected in estrus must be improved (Macmillan and Peterson, 1993;

Van Cleef et al., 1996; Stevenson et al., 2003; Chapter 9). However, treatments designed to synchronize ovulatory follicle growth and increase estrous detection rate must not jeopardize pregnancy following TAI.

Estradiol treatment of progestin-treated cattle has been shown to induce a new follicular wave, regardless of stage of the estrous cycle at the time of treatment (Bó et al., 1995a,b), and a reduced dose of estradiol cypionate (ECP) at progestin withdrawal induced estrous behavior and synchronous LH release and ovulation (Colazo et al., 2003b, Chapter 5). However, estradiol treatment of previously inseminated heifers has been reported to reduce pregnancy rate following TAI (Cutaia et al., 2002; Chapter 9). An injection of progesterone at CIDR insertion (13 ± 1 d after TAI) did not affect pregnancy rate following TAI, but the incidence of behavioural estrus and fertility in nonpregnant heifers displaying estrus were decreased (Chapter 9).

Gonadotrophin releasing hormone (GnRH) may be an alternative for synchronizing follicular wave emergence in cattle with unknown pregnancy status. It has also been suggested that GnRH treatment during diestrus would result in most cattle having three follicular waves (Clark et al., 1998), which may potentially increase fertility at the subsequent estrus (Townson et al., 2002). However, when GnRH was given to cows between Days 12 and 16 of the estrous cycle, cycle length was significantly longer than in control cows (Macmillan et al., 1985).

Recently, pregnancy rate was reported to be higher in cattle resynchronized with a used progesterone intravaginal insert (CIDR) than in those fed melengestrol acetate (MGA) for 7 d (Stevenson et al., 2003; Chapter 9). Because of variable MGA clearance rate from the digestive tract, a shorter-term MGA regimen warrants further investigation.

The objectives of the present studies were to evaluate the effects of ECP, GnRH or progesterone treatment during diestrus in non-bred beef cows on ovarian follicular dynamics, CL function, estrus, and ovulation, and to determine whether these treatments, along with MGA or a used CIDR, would increase estrous and pregnancy rates in beef heifers not conceiving following TAI.

10.3 Materials and Methods

10.3.1 Experiment 1

Nonlactating, nonpregnant crossbred beef cows (n = 41), 3 to 9 yr of age were previously synchronized with an estradiol-CIDR based protocol and ovulation time was determined by twice-daily ultrasonography (Colazo et al., 2005, Chapter 7). On Day 12.3 ± 0.7 d (mean ± SD; Day 0) after ovulation, all cows received a once-used CIDR insert (Bioniche Animal Health, Belleville, ON, Canada) that had been washed and disinfected as previously described (Colazo et al., 2004b, Chapter 8). At the same time, cows were randomly allocated to concurrently receive one of the following four treatments: no further treatment (Control; n = 10), 1 mg of estradiol cypionate (ECP; Pharmacia Animal

Health, Orangeville, ON, Canada; n=11), 100 μg of gonadotrophin releasing hormone (GnRH; Cystorelin, Merial Can Inc., Victoriaville, PQ, Canada; n=11) or 150 mg of progesterone in 2 mL canola oil (Sigma Chemical Co., St. Louis, MO, USA; n=11). The ECP, GnRH and progesterone were all given as intramuscular (im) injections. On Day 7, CIDR inserts were removed from all cows, and half in each group received 0.5 mg im of ECP.

Cows were monitored once daily (Days 0 to 8) by ultrasonography (Aloka SSD 500 with a 7.5 MHz linear-array transducer; ISM Inc., Edmonton, AB, Canada) to determine ovarian follicular dynamics, and twice daily (Days 8 to 12) to determine the time of ovulation. The diameter of the CL and all follicles ≥3 mm were measured and recorded (Pierson and Ginther, 1984). The day of emergence of a follicular wave was defined as the day that the dominant follicle was first identified at a diameter of 4 mm (Ginther et al., 1989b). When follicular wave emergence did not occur during the observation period, day of follicular wave emergence prior to treatment was estimated from the size of the dominant follicle at the time of treatment. Ovulation was confirmed by the disappearance of a large (>10 mm) follicle that had been detected at the previous examination.

Visual observation for estrous behaviour was done three times daily (40 min each) from Days 8 to 12. Blood samples were collected by coccygeal venipuncture at 24-h intervals (Days 1 to 9) into an evacuated tube containing heparin. Blood samples were centrifuged at 1500 x g for 20 min and plasma separated and stored at –20 °C until

assayed for progesterone by an enzyme-immunoassay (Del Vecchio et al., 1995). The intra- and inter-assay coefficients of variation were 7.1 and 10.2%, respectively.

10.3.2 Experiment 2

Angus and Angus-cross heifers (n=675), 12 to 15 mo of age and weighing approximately 320 to 400 kg, were used between April and June, 2003. All heifers were managed in a drylot (8 pens), fed a barley-silage based diet and were synchronized with an MGA-based protocol for TAI. Thirteen or 14 d after TAI, heifers were fed melengestrol acetate (MGA; Pharmacia Animal Health; 0.5 mg/head/d) for 6 d (designated Days 0 to 5). On Day 0, heifers were randomly allocated to one of two groups to receive either no further treatment (Control) or 100 μg im of GnRH at the first feeding of MGA. On Day 7 (2 d after the last feeding of MGA), half of the heifers in each group received 0.5 mg im of ECP in a 2 by 2 factorial design.

Estrus detection was done as in Experiment 1. Heifers observed in estrus were reinseminated 6 to 12 h later by a single technician, using commercial frozen-thawed semen from one of two bulls, balanced across treatment groups. Ultrasonographic pregnancy diagnosis was conducted approximately 28 d after TAI in heifers not seen in estrus following resynchronization, and approximately 55 d after the second AI in those that were detected in estrus and reinseminated.

10.3.3 Experiment 3

Angus and Angus-cross heifers (n=317) were managed (2 pens), fed, synchronized and submitted to TAI as described for Experiment 2. On Day 0 (13 to 14 d after TAI), heifers were randomly allocated to either be fed MGA (as in Experiment 2) or receive a once-used CIDR insert for 7 d. On Day 7, CIDR inserts were removed and half of the CIDR- and MGA-treated heifers were given 0.5 mg im of ECP. Estrus detection, reinsemination and pregnancy diagnosis were done as in Experiment 2.

10.3.4 Statistical Analyses

Throughout this study, data are reported as means ± SD. A one-way ANOVA was used to compare the intervals from treatment during diestrus to follicular wave emergence, and from CIDR removal to estrus and ovulation among treatments (Experiment 1), and from TAI to reinsemination among treatment groups in Experiments 2 and 3. Means were compared with the protected LSD test and equality of variances was compared by Bartlett's test. In Experiment 1, the number of follicles by day was categorized according to follicle size; small (3 to 5 mm), intermediate (6 to 9 mm) and large (10 mm or greater). Data for each size category were analyzed using generalized estimating equations (GEE; PROC GENMOD). The main-effects model was assessed for first-order interactions where treatment and day remained in the model, with $P<0.05$ for both variables in the GEE analysis. Data for CL and follicle diameters and plasma progesterone concentrations were analyzed by the Proc Mixed Model procedure in SAS

using autoregressive-1 (AR-1) as the covariate structure for repeated measurements (Littel et al., 1998). In Experiments 2 and 3, associations between pregnancy status following TAI and rebreeding and treatment groups were analyzed using a GEE (PROC GENMOD) method to account for clustering due to pen effect (Experiment 2). Model specifications included a binomial distribution, logit link function, repeated statement with subject equal to pen, and an exchangeable correlation structure. Treatments were analyzed as a fixed effect with two categories. Proportional data were compared by Chi-square test. All data were analyzed using a statistical computer software program (SAS Version 8.2 for Windows; SAS Institute, Cary, North Carolina, USA).

10.4 Results

10.4.1 Experiment 1

Follicle wave emergence following treatment on Day 0 was most synchronous ($P<0.0001$), and diameter of the dominant follicle at CIDR removal on Day 7 was least variable ($P=0.01$) in the GnRH group (Table 10.1). The largest follicle present on Day 0 was the ovulatory follicle in 50.0, 36.4, 18.2, and 54.5% of cows in the control, ECP-, GnRH-, and progesterone-treated groups, respectively ($P=0.3$). Ovulation occurred 48 h after GnRH treatment in eight of 11 cows; a new follicular wave emerged 24 h before ovulation in seven of these cows. However, in one cow, the largest subordinate follicle became the dominant follicle.

Diameters of the dominant follicles (≥9mm), present on Day 0 that regressed or continued growing after treatment are shown in Figure 10.1. In those cows in which follicle regression occurred, regression rate (from Days 0 to 7) was not different among treatments (overall 0.59 ± 0.24 mm per day; P=0.7). However, the regressing dominant follicle was smaller (P<0.05) in the ECP and control groups than the GnRH group from Day 4 until CIDR removal (Figure 10.1A). Dominant follicles that did not regress and continued growing tended to grow more slowly in ECP- and progesterone-treated cows than in control cows (0.6 ± 0.5 and 0.5 ± 0.4 versus 1.0 ± 0.4 mm per day, respectively, P=0.09; Figure 10.1B).

The number of small follicles (3 to 5 mm) was affected by day (P<0.0001) and tended to be affected by treatment (P<0.07; Figure 10.2). The number of intermediate-sized follicles (6 to 9 mm) tended to be affected by day (P=0.08) and there was a day by treatment interaction (P<0.06). The diameter of the CL was affected by day (P<0.0001) and tended to be affected by treatment (P<0.1) and there was a treatment by day interaction (P=0.06; Figure 10.3A). However, there was only an effect of day (P<0.0001) on plasma progesterone concentrations (Figure 10.3B).

Intervals from CIDR removal to estrus and ovulation were not affected by treatments on Day 0 (P=0.2; Table 10.2). However, 10/43 cows were not seen in estrus after CIDR removal (13.6 % received ECP and 33.3% received no treatment on Day 7; P<0.1). The interval from CIDR removal to ovulation tended to be affected (P=0.07) by administration of ECP at CIDR removal (68.7 ± 9.2 versus 75.0 ± 12.2 h for ECP and

control groups, respectively) and was affected (P<0.05) by the interaction between treatments at CIDR insertion and removal. Cows given either GnRH or ECP at CIDR insertion and no further treatment at CIDR removal had a longer interval from CIDR removal to ovulation than cows in the other 6 treatment groups (Table 10.2). One cow in the control group and one cow in the progesterone-treated group did not ovulate by Day 12 of the experiment.

Table 10.1. Mean (± SD) day of follicular wave emergence, and diameter of the dominant follicle at CIDR removal and prior to ovulation in beef cows given a CIDR (Control), or a CIDR and concurrently treated with 1 mg of estradiol cypionate (ECP), 100 μg of GnRH (GnRH) or 150 mg of progesterone (P) on Day 0 (Day 12.3 ± 0.7 of diestrus). At CIDR removal (Day 7), half of the cows in each group received 0.5 mg of ECP.

	Control	ECP	GnRH	P
No. cows	10	11	11	11
Day of follicular wave emergence				
Mean	0.2	0.7	0.8	-0.2
SD[1]	3.7	4.1	0.8	3.3
Range	-5 to 6	-6 to 5	-1 to 2	-6 to 5
Diameter (mm) of dominant follicle				
At CIDR removal (Day 7)				
Mean	14.4	12.4	13.1	14.3
SD[2]	4.3	3.2	1.4	2.7
Range	7 to 20	8 to 19	12 to 17	10 to 19
Just prior to ovulation				
Mean	17.7[a]	15.3[b]	15.6[b]	16.4[ab]
SD	2.6	2.0	1.4	2.5
Range	14 to 21	13 to 20	14 to 18	13 to 21

[ab] Means within the row tended to differ (P=0.08)
[1] Standard deviations within the row differed (P<0.0001)
[2] Standard deviations within the row differed (P=0.01)

Table 10.2. Estrus and ovulation rates and mean (± SD) intervals from CIDR removal to estrus and ovulation in beef cows given a CIDR (Control), or a CIDR plus 1 mg of estradiol cypionate (ECP), 100 μg of GnRH (GnRH) or 150 mg of progesterone (P) on Day 0 (Day 12.3 ± 0.7 of diestrus). At CIDR removal (Day 7), half of the cows in each group received 0.5 mg of ECP.

	Control	ECP	GnRH	P
Estrus				
No further treatment				
No. cows	2/5	3/5	5/6	4/5
Interval (h)				
Mean	32.0	50.7	48.0	35.0
SD	0.0	4.6	11.3	10.0
Range	32	48 to 56	38 to 64	24 to 48
Treated with ECP				
No. cows	4/5	5/6	5/5	5/6
Interval (h)				
Mean	40.0	35.2	40.0	36.8
SD	9.2	7.7	11.3	9.1
Range	32 to 48	28 to 48	32 to 56	28 to 56
Ovulation[1]				
No further treatment				
No. cows	4/5	5/5	6/6	5/5
Interval (h)				
Mean	66.0	81.6	82.0	67.2
SD	6.9	10.0	14.0	6.6
Range	60 to 72	72 to 96	72 to 108	60 to 72
Treated with ECP				
No. cows	5/5	6/6	5/5	5/6
Interval (h)				
Mean	69.6	68.0	67.2	70.0
SD	10.0	9.8	10.7	9.3
Range	60 to 84	60 to 84	60 to 84	60 to 84

[1] Interval from CIDR removal to ovulation; effect of treatment at CIDR insertion (P=0.2), effect of treatment at CIDR removal (P=0.07) and their interaction (P=0.05).

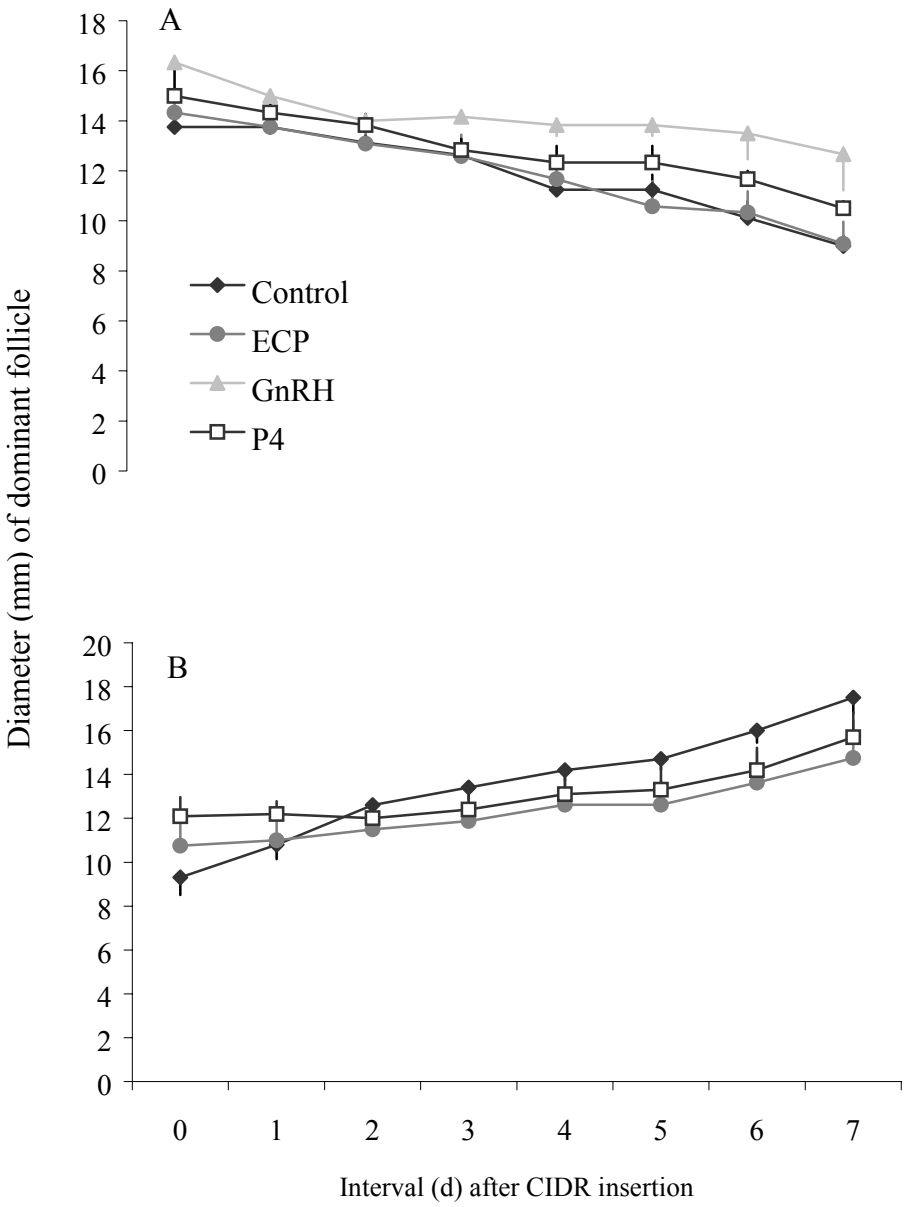

Figure 10.1. Mean diameter of dominant follicles that regressed (A) or continued growing (B) in beef cows given a once-used CIDR insert (Control) 12.3 ± 0.7 d after ovulation (designated as Day 0), or a once-used CIDR plus 1 mg of ECP (ECP), 100 μg of GnRH (GnRH), or 150 mg of progesterone (P). A) Diameter of dominant follicles that regressed was smaller in the ECP (n=7) and control (n=5) groups than in the GnRH (n=3) group from Day 4 until CIDR removal ($P<0.05$). B) Dominant follicles that did not regress after treatment tended to grow more slowly in ECP- (n=4) and P- (n=6) treated cows than in control (n=5) cows (0.6 ± 0.5, 0.5 ± 0.4, and 1.0 ± 0.4 mm per day, respectively; $P=0.09$). There was no dominant follicle in the GnRH-treated group because it either ovulated (n=8) or regressed (n=3).

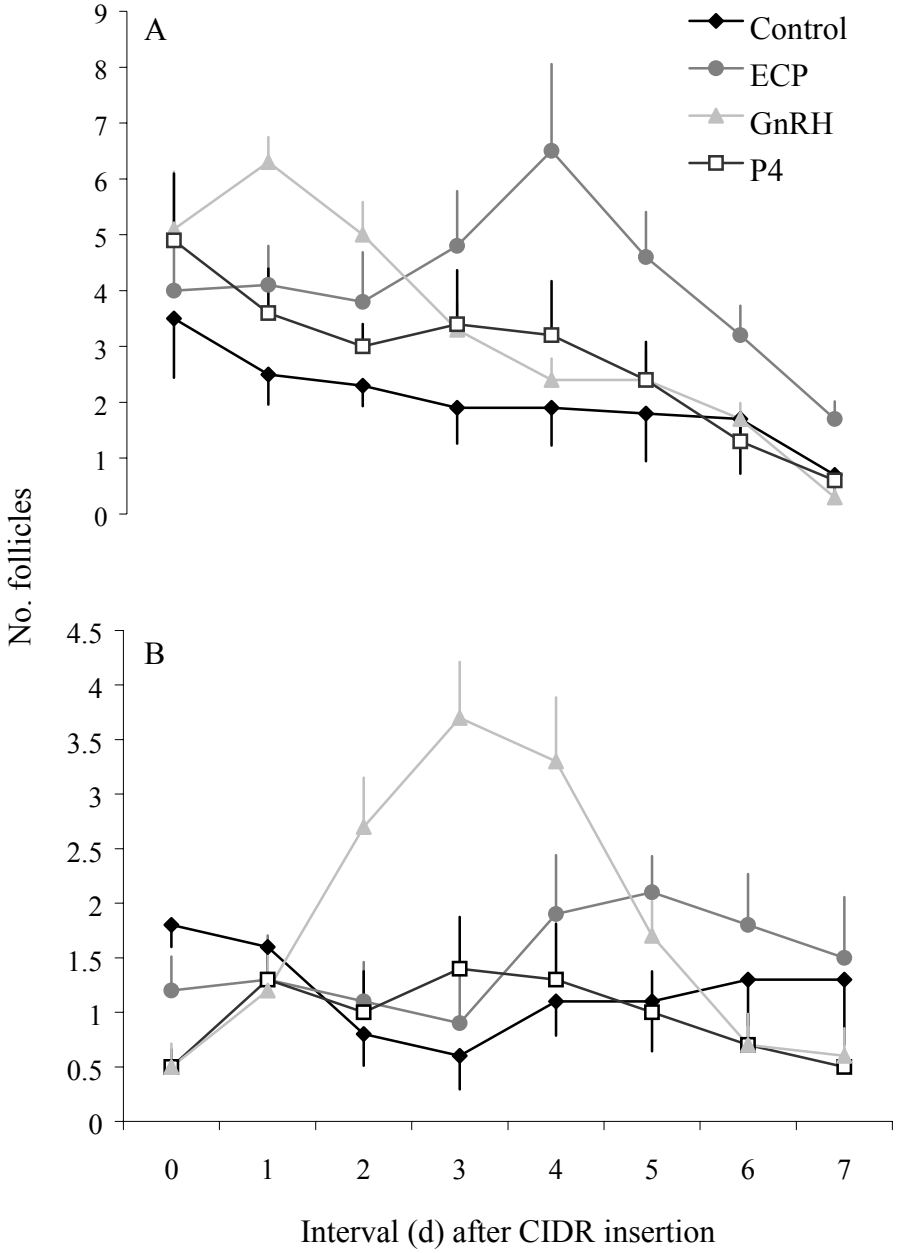

Figure 10.2. Numbers of small follicles (3 to 5 mm; A) and intermediate follicles (6 to 9 mm; B) in beef cows given a once-used CIDR insert (Control) or a once-used CIDR plus 1 mg of estradiol cypionate (ECP), 150 mg of progesterone (P) or 100 μg of GnRH (GnRH) 12.3 ± 0.7 d after ovulation (designated as Day 0). The number of small follicles was affected by day ($P<0.0001$) and treatment ($P=0.07$). The number of intermediate follicles tended to be affected by day ($P=0.08$) and a treatment by day interaction ($P<0.06$).

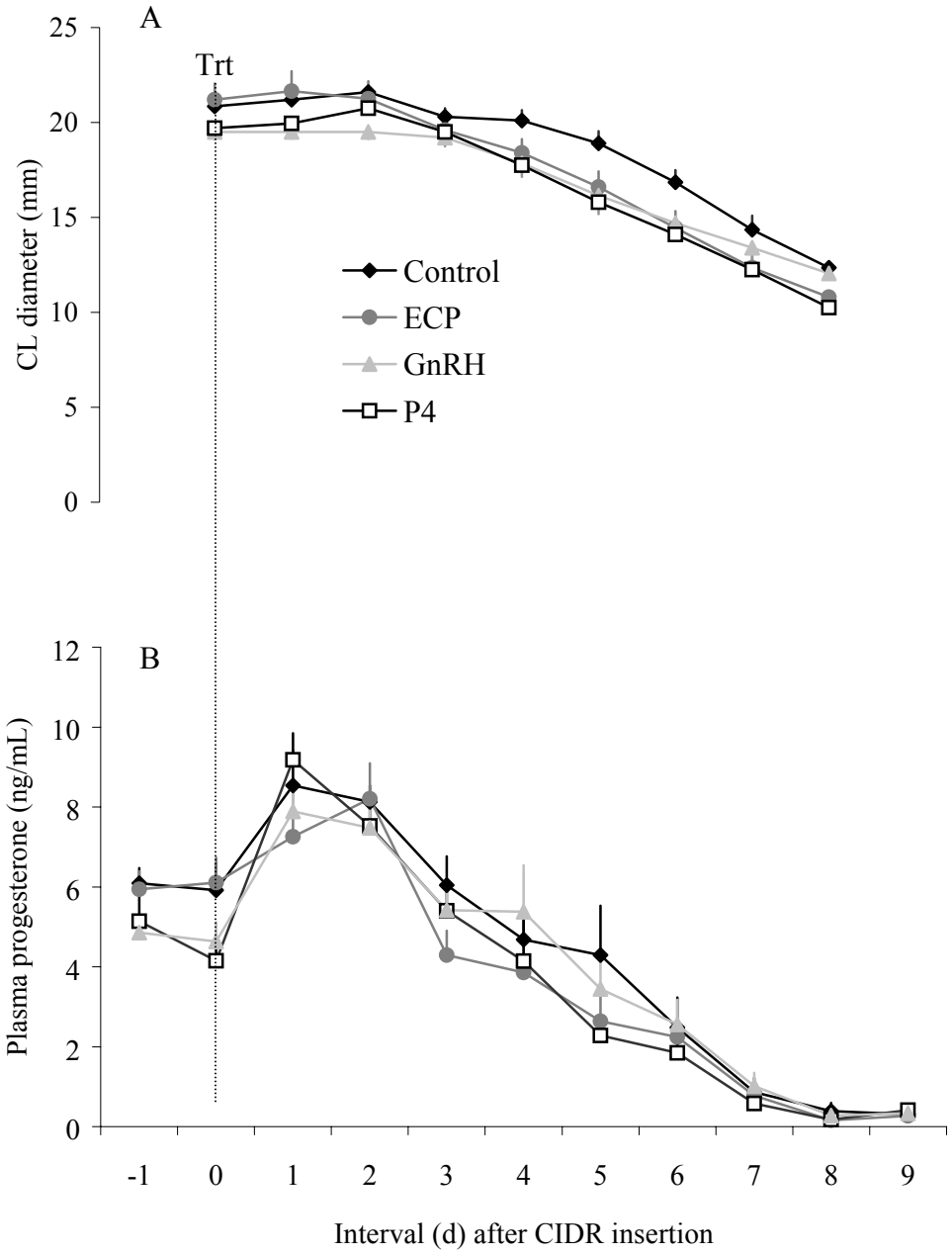

Figure 10.3. CL diameter (A) and plasma progesterone concentrations (B) in beef cows given a once-used CIDR insert 12.3 ± 0.7 d after ovulation (designated as Day 0), with no further treatment (Control), or a once-used CIDR plus 1 mg of ECP (ECP), 100 μg of GnRH (GnRH), or 150 mg of progesterone (P). On Day 7, all CIDR inserts were removed and half of the cows received 0.5 mg of ECP. The diameter of the CL was affected by day ($P<0.0001$) and tended to be affected by treatment ($P<0.1$) and a treatment by day interaction ($P=0.06$). There was an effect of day ($P<0.0001$) on progesterone concentrations.

10.4.2 Experiment 2

Pregnancy rate following TAI was 37.8% and was not affected by treatments on Day 0 (P=0.7) or Day 7 (P=0.3). Overall, 419 heifers were diagnosed ultrasonically to be not pregnant following TAI. Treatment with GnRH on Day 0 had no effect on estrous rate (P=0.5), whereas ECP treatment on Day 7 tended to increase (P<0.1) estrous rate (69.6 versus 62.7%; Table 10.3). Interval from Day 7 (ECP treatment) to reinsemination was more synchronous (P<0.05) in heifers in the GnRH and GnRH/ECP groups (2.3 ± 1.2 and 1.9 ± 1.1 d, respectively) than in control heifers, whether they received ECP (2.2 ± 1.4 d) or not (2.1 ± 1.4 d).

Table 10.3. Reproductive outcome of nonpregnant heifers resynchronized with MGA starting 13 or 14 d (Day 0) after timed-AI (TAI). All heifers (n=675) were fed MGA (0.5 mg/head/day) for 6 d and were allocated to receive 100 μg of GnRH or nothing on Day 0 (first day of MGA feeding) and 0.5 mg of ECP or nothing on Day 7.

	Control	GnRH	Control/ECP	GnRH/ECP
No. heifers nonpregnant to TAI	108	107	101	103
Resynchronization				
No. in estrus	67	68	74	68
Estrus rate (%)	62.0[b]	63.5[b]	73.3[a]	66.0[b]
No. pregnant to reinsemination	30	35	39	27
Conception rate (%)	44.8	51.5	52.7	39.7
Pregnancy rate (%)[1]	27.7	32.7	38.6	26.2

[ab] Within a row, percentages without a common superscript tended to differ (P=0.1).
[1] Based on numbers of heifers subsequently found to be not pregnant following TAI.

10.4.3 Experiment 3

Pregnancy rate following TAI was 38.7% and was not affected by treatments on Day 0 (P=0.5) or Day 7 (P=0.2). Overall, 195 heifers were diagnosed ultrasonically to be not pregnant following TAI (Table 10.4). There was no effect of treatment on estrous rate (P=0.4; overall, 73.8%). However, heifers receiving a CIDR only had a more synchronous (P<0.003) interval from Day 7 (CIDR removal) to reinsemination (2.2 ± 0.7 d) than those in the other three groups (2.6 ± 1.3, 2.6 ± 1.3, and 2.4 ± 1.4 d for CIDR/ECP, MGA, and MGA/ECP groups, respectively). Furthermore, heifers receiving a CIDR tended (P<0.1) to have a higher conception rate (68.8 versus 56.7%) and had a higher (P<0.04) pregnancy rate (54.1 versus 39.2%) than those fed MGA. Treatment with ECP on Day 7 had no effect on conception (P=0.4) or pregnancy (P=0.8) rates.

Table 10.4. Reproductive outcome in nonpregnant heifers resynchronized with MGA or a used-CIDR starting 13 or 14 d (Day 0) after timed-AI (TAI). Heifers (n=317) were given MGA (0.5 mg/head/day) for 6 d or a once-used CIDR for 7 d and were subsequently given 0.5 mg of ECP or nothing on Day 7.

	MGA	MGA/ECP	CIDR	CIDR/ECP
No. heifers nonpregnant to TAI	56	41	51	47
Resynchronization				
No. in estrus	38	29	37	40
Estrus rate (%)	67.8	70.7	72.5	85.1
No. pregnant to reinsemination	22	16	28	25
Conception rate (%)	57.9[a]	55.2[a]	75.7[b]	62.5[b]
Pregnancy rate (%)[1]	40.4[c]	39.0[c]	54.9[d]	53.2[d]

[ab] Within a row, percentages without a common superscript tended to differ (P=0.1)
[cd] Within a row, percentages without a common superscript were different (P=0.04)
[1] Based on numbers of heifers subsequently found to be not pregnant following TAI.

10.5 Discussion

Gonadotrophin releasing hormone was the only treatment that effectively synchronized follicular wave emergence in cows treated with a CIDR insert during diestrus (12.3 ± 0.7 d after ovulation). Administration of GnRH induced ovulation and emergence of a new follicular wave in more than 80.0% of cows, but it did not alter estrous cycle length, suggesting that GnRH maybe useful in the resynchronization of cows following TAI. In a previous study, treatment with a GnRH agonist on Day 11 of the estrous cycle increased estrous cycle length, primarily by reducing the incidence of cycles less than 20 d in length (Macmillan et al., 1985). However, other studies involving treatment with either GnRH (Macmillan and Thatcher, 1991) or hCG (Price and Webb, 1989) during diestrus revealed no effect on estrous cycle length.

In cattle resynchronized with progestins, fertility could be compromised by prolonged growth and dominance of the ovulatory follicle in those that had spontaneous luteal regression prior to progestin withdrawal. Follicles with prolonged dominance ("persistent follicles") have been extensively described in the literature (Sirois and Fortune, 1990; Savio et al., 1992; 1993; Stock and Fortune, 1993; Mihm et al., 1994; Kojima et al., 2003); oocytes from these follicles had a lower fertility (pregnancy rate) than those from "healthy" dominant follicles (Savio et al., 1992; Stock and Fortune, 1993; Mihm et al., 1994). Two recent studies showed that fertility tended to be (65.7 versus 44.1%) (Chapter 9), or was higher (60.0 versus 33.3%) (Stevenson et al., 2003) in untreated control heifers than in heifers treated with a used CIDR following TAI. This can be overcome by inducing a new follicular wave at the time of initiating progestin

treatment. In addition, it has been suggested that cattle with three follicular waves have higher fertility than those with two follicular waves (Townson et al., 2002) and treatments that result in all animals having three follicular waves should increase fertility. Administration of GnRH during diestrus significantly increased the proportion of cattle with three-waves (Clark et al., 1998) and induced a new follicular wave in high proportion (80%) of cows in Experiment 1. However, GnRH treatment did not increase conception rate in reinseminated heifers (in Experiment 2), even though the synchrony of estrus suggested that GnRH had synchronized follicle wave emergence. Heifers may not have been as good model as cows to test this hypothesis because of the previously reported lower ovulatory response following GnRH treatment during diestrus in heifers (Pursley et al., 1995; Martínez et al., 1999).

Administration of either 1 mg of ECP or 150 mg of progesterone during diestrus did not effectively synchronize follicular wave emergence in this study; the range in the interval from treatment to follicular wave emergence for both groups did not differ from that in the control group. Colazo et al. (2003b, Chapter 5) have previously reported that follicular wave emergence was quite variable in CIDR-treated beef heifers following treatment with 1 mg of ECP. In addition, the administration of 150 mg of progesterone concurrent with insertion of a used CIDR 13 to 14 d after TAI in heifers resulted in reduced estrous behaviour, conception and pregnancy rates at the subsequent estrus (Chapter 9).

Circulating FSH concentrations and LH pulse amplitude have been shown to decrease following administration of estradiol and progesterone (Price and Webb, 1988; Bó et al., 1995b), resulting in the regression of FSH- and LH-dependent follicles (Bó et al., 1995b). Since progesterone affects LH pulse frequency (Ireland and Roche, 1982), progesterone treatment is most likely to affect only LH-dependent follicles (Adams et al., 1992b), i.e. dominant follicles ≥9 mm of diameter (Ginther et al., 2001). Although all cows had a dominant follicle ≥9 mm of diameter on Day 0 in Experiment 1, injection of 150 mg progesterone (and insertion of a used CIDR) resulted in emergence of a new follicular wave in only 45.5% of cows. In non-responding cows, growth rate of the dominant follicle was reduced, suggesting that progesterone treatment may have been partially effective in suppressing LH release. In other studies, the induction of atresia of persistent follicles has been successfully achieved with short-term increases in progesterone, presumably due to decreased LH pulse frequency (Ireland and Roche, 1982; Price and Webb, 1988), and administration of 200 mg progesterone in cows with persistent follicles resulted in a relatively synchronous emergence of a new follicular wave 3.5 ± 0.3 d later (Anderson and Day, 1994). In another study, treatment with 100 mg progesterone (in oil) or a new CIDR insert for 24 h induced atresia of persistent follicles in *Bos indicus* cattle (Cavalieri et al., 1998), suggesting that persistent follicles may be more sensitive to short-term changes in LH pulse frequency than "healthy" dominant follicles.

Resynchronization of estrus in cattle must not disrupt ongoing pregnancies from a previous insemination. In this regard, the use of estradiol to initiate a follicular wave emergence or increase estrous activity is of some concern in cattle with unknown pregnancy status. In Experiments 2 and 3, there was no indication that any of the resynchronization protocols affected pregnancy rate following TAI. Similarly, reduced dosages of estradiol benzoate or estradiol cypionate to synchronize follicular wave emergence (Macmillan et al., 1997; Stevenson et al., 2003) and ovulation (Stevenson et al., 2003; El-Zarkouny and Stevenson, 2004) in resynchronized dairy and beef cows did not seem to affect pregnancy rate following TAI. Conversely, the same dosages of estradiol benzoate (Cutaia et al., 2002) or estradiol-17β (Chapter 9) to resynchronize beef heifers was associated with reduced pregnancy rates following TAI. The diameters of the CL present at the time of treatment, and plasma progesterone concentrations, were not affected by any of the treatments in CIDR-treated cows in Experiment 1. Plasma progesterone concentrations in CIDR-treated cows were not different between those that received GnRH or no further treatment, even though GnRH induced an accessory CL in 8 out of 11 cows. In addition, treatment with GnRH during diestrus may affect morphology of the CL and have a varied effect on progesterone production (for review see Twagiramungu et al., 1995b). Although plasma progesterone concentrations were not affected, the number of large luteal cells and volume of the CL were increased following buserelin treatment (Twagiramungu et al., 1995a).

Follicle numbers tended to be affected by treatment at CIDR insertion in Experiment 1; numbers of small follicles increased 1 and 4 d after treatment in GnRH- and ECP-treated cows, respectively, but declined following treatment with injectable progesterone. However, GnRH induced ovulation between 24 and 48 h after treatment in 8 of 11 cows, suggesting that the number of small follicles increased before ovulation, likely due to increased concentrations of FSH following GnRH treatment (Chenault et al., 1990), rather than ovulation of the dominant follicle (Adams et al., 1992a). Indeed, emergence of the new follicular wave after ovulation occurred in only one GnRH-treated cow. However, ovulation or atresia of the large dominant follicle is necessary to prolong the growth of FSH-dependent follicles (Adams et al., 1993a). In GnRH-treated cows, the number of medium-sized follicles increased between Days 2 and 4 (peak on Day 3), which may reflect the continued development of follicles emerging as a result of the more transient increase in FSH following GnRH treatment. In this regard, Macmillan and Thatcher (1991) also reported that a single injection of a GnRH agonist on Day 12 increased the average number of medium-sized follicles 1 to 6 d later.

High rates of both synchronous estrus and conception are needed to optimize pregnancy rate in nonpregnant cattle returning to estrus following AI. In previous studies that used only progestins to resynchronize estrus, there was a clear need to increase the number of nonpregnant cattle showing estrus following progestin removal (Van Cleef et al., 1996; Stevenson et al., 2003; Chapter 9). In two previous studies (Van Cleef et al., 1996; Chapter 9), reinsertion of a used CIDR resulted in approximately 60.0 to 80.0% of the non-pregnant heifers detected in estrus over a 5-d period, and in yet another study, the

proportions of non-pregnant heifers that returned to estrus following resynchronization with either MGA or CIDR treatments were 65.9 and 63.6%, respectively (Stevenson et al., 2003). To our knowledge, there has been only one experiment in which 90.0% of heifers showed behavioural estrus after progestin withdrawal (Chapter 9; Experiment 2).

Although estrus was detected by visual observation in most of the previous studies, the proportion of non-pregnant cattle detected in estrus was not greatly improved when a HeatWatch^{TM} system was used (70.6 and 84.0%, Stevenson et al., 2003 and Chapter 9, respectively). We hypothesized that the inclusion of estradiol at the time of progestin removal would increase the proportion of non-pregnant cattle observed in estrus. In Experiment 1, treatment with 0.5 mg ECP at CIDR removal tended to increase the proportion of cows observed in estrus; estradiol at CIDR removal also tended to shorten the interval from CIDR removal to ovulation, with a more profound effect in cows given either GnRH or ECP at CIDR insertion. Estradiol treatment also tended to increase the number of heifers seen in estrus in heifers fed MGA in Experiment 2. Although estradiol treatment had no effect on estrous detection rate in Experiment 3, numerically more CIDR-treated heifers were seen in estrus. However, due to a reduced conception rate, pregnancy rate (the product of estrus detection and conception rates) was not improved in CIDR-treated heifers given ECP (compared to CIDR-treated heifers). Therefore, there was no benefit to giving estradiol after progestin withdrawal in these experiments, which is in agreement with our previous study (Chapter 9).

Overall, conception and pregnancy rates were higher in nonpregnant heifers resynchronized following TAI with a used-CIDR as compared to feeding MGA (Experiment 3). We have previously reported that conception and pregnancy rates in heifers fed MGA for 6 d (49.6 and 40.4%, respectively) were lower than in those given a CIDR for 7 d (65.1 and 61.4%, respectively) or untreated-controls (62.2 and 54.9%, respectively; Chapter 9). A shorter-term MGA regimen and the attempt to synchronize ovarian follicular dynamics did not improve fertility in MGA-fed heifers in the present study. In Experiment 2, administration of GnRH at first feeding of MGA did not improve conception rate, despite a more synchronous estrus. Similarly, Stevenson et al. (2003) reported that 0.5 mg ECP treatment at first feeding and after cessation of MGA feeding (13 and 20 d after AI, respectively) did not increase conception rates over that in untreated-controls. Although estradiol-17β and progesterone treatment at the initiation of feeding MGA on Day 13, in a previous study, resulted in an increase of 14.0% in conception rate of over untreated-MGA fed heifers, fertility was still lower than in untreated-control (9.0%) and estradiol-CIDR-treated (20.0%) heifers (Chapter 9; Experiment 2). Therefore, MGA would not appear to be a good alternative for the resynchronization of returns to estrus in time-inseminated cattle.

Insertion of used CIDR inserts into previously inseminated cattle resulted in a decreased numbers of days necessary for estrus detection (Chapter 9). Thus, it was a simple approach to the resynchronization of estrus in nonpregnant cattle, with acceptable fertility in reinseminated heifers (Chapter 9). In this regard, Macmillan and Peterson, (1993) reported conception rates of 65.6% in cows resynchronized with a new CIDR

insert. It is noteworthy, that in the present study, fertility at the resynchronized estrus in progestin-treated heifers was not compared to that in untreated-control heifers. However, Chenault et al. (2003) reported conception rates of 26.7% in lactating dairy cows resynchronized with a new CIDR which was not different than in untreated-controls (30.9%). Conversely, Stevenson et al. (2003) reported lower conception rates in heifers receiving a used-CIDR to resynchronize estrus (33.3%) as compared to untreated-controls (60.0%). In a previous study, we found that conception rate in CIDR-treated heifers was numerically lower than in untreated-control heifers (62.5 versus 76.7%), but the difference was not significant due to limited power to detect a difference (Chapter 9; Experiment 1). Based on these reports, we could assume that CIDR treatment to resynchronize estrus in previously inseminated cattle might result in marginally lower conception rates. However, the reduced amount of time required for estrus detection may compensate for this reduction in fertility.

In summary, GnRH treatment during diestrus synchronized follicular wave emergence in noninseminated cows and shortened the estrus detection period in nonpregnant heifers resynchronized following TAI. In contrast, 1.0 mg ECP or 150 mg progesterone given at CIDR insertion did not synchronize follicular wave emergence in nonlactating beef cows, but ECP treatment following progestin removal tended to shorten and synchronize the interval to onset of estrus. Feeding MGA resulted in lower conception and pregnancy rates than a used-CIDR insert for resynchronization; administration of GnRH at first feeding of MGA did not improve fertility. Overall, treatment with ECP at or after progestin withdrawal tended to increase the estrous

detection rate, but numerically decreased conception rate in CIDR-treated heifers. None of the treatment protocols affected CL function, estrous cycle length or ongoing pregnancies to timed-AI.

11.0 GENERAL DISCUSSION

Reproductive physiologists have been working on the development of estrus synchronization programs that overcome the problems and limitations associated with visual estrus detection. In addition, the synchronization of estrus will increase the use of artificial insemination (AI) with less intensively managed cattle. Timed artificial insemination (TAI) has a direct impact on cost-efficiency by reducing time and labor associated with estrus detection. However, to make TAI a widely used technique, hormonal treatments used in estrus synchronization systems need to be commercially available, economical and result in acceptable pregnancy rates. The studies reported in this thesis had as an objective to develop alternative estrus synchronization and resynchronization protocols to facilitate and increase the use of AI in beef cattle. Nevertheless, several factors such as oocyte competence, fertilization rate, CL function, and embryo development may affect fertility following estrus synchronization and TAI. The following sections are intended to review studies reported herein, and to discuss factors affecting fertility that should be considered in future studies.

11.1 Synchronizing follicular wave emergence for estrus synchronization schemes

The effects of different estradiol preparations on the pattern of gonadotrophin release and ovarian follicular dynamics have been previously described. It was reported that 5 mg of estradiol-17β (E-17β) resulted in a transient decrease in FSH concentrations, followed by a transient increase in FSH, and synchronous follicular wave emergence 4.3 d after treatment (Bó et al., 1995b). Length of suppression of endogenous FSH release and the interval to follicular wave emergence depended on estradiol preparation and dose (Martinez et al., 2003a). Slow absorption, prolonged elevations in plasma concentrations of estradiol and subsequently delayed and asynchronous follicular wave emergence were reported in cattle treated with large doses (5-10 mg) of estradiol esters (see Chapters 5 and 7). In this regard, Caccia and Bó (1998) reported that 2.5 mg estradiol benzoate (EB) induced more synchronous emergence of a new follicle wave than a dose of 5 mg. To our knowledge, the effects of reduced doses of estradiol cypionate (ECP) had been reported in only one study (Thundathil et al., 1997), and the effects of reduced doses of estradiol valerate (EV) had not been studied. Thus, ECP was the only estradiol preparation commercially available in North America and EV was under consideration for licensing along with the norgestomet implant called Crestar^{TM}, so we elected to study the use of these esters in TAI protocols. We hypothesized that administration of reduced doses (1-2 mg) of ECP or EV would result in synchronous follicular wave emergence regardless of stage of the estrous cycle at the time of treatment. However, present studies revealed that in beef heifers, 1 mg of ECP was less precise in synchronizing follicular wave emergence

than 5 mg of E-17β, while in beef cows, a dose of 1 or 2 mg of EV resulted in synchronous follicular wave emergence.

We confirmed that EV, an estradiol ester, is efficacious in synchronizing follicle wave emergence in cattle, providing the appropriate dose is used. This is in agreement with previous studies in which reduced doses of EB, another estradiol ester, was investigated. In contrast, the 1 mg dose of ECP utilized in studies described in Chapter 5 did not result in synchronous follicular wave emergence, which is consistent with the findings of Thundathil et al. (1997). Lopes (2000b) showed that a dose of 1 mg ECP resulted in circulating E-17β plasma concentrations of 10-15 pg/mL, which would appear to be required to suppress circulating FSH concentrations sufficiently to induce follicle atresia, and then emergence of a new follicular wave following a transient FSH surge (Diskin et al., 2002). Hence, we do not consider it necessary to increase the dose of ECP to improve its efficacy in synchronizing follicle wave emergence. On the contrary, a higher dose of ECP may result in a more delayed and asynchronous emergence of the new follicular wave because of the duration of increase plasma estradiol concentrations.

Gonadotrophin releasing hormone (GnRH) has been used extensively to synchronize follicular dynamics. A gonadotrophin surge (Kaltenbach et al., 1974; Chenault et al., 1990; Martinez et al., 2003b) and ovulation in approximately 85% of cows and 54% of heifers (Pursley et al., 1995) following administration of GnRH has been described. Ovulation of a selected dominant follicle will ensure the emergence of a follicular wave, on average 2 d after treatment. Whether a proportion of animals that do

not ovulate in response to GnRH treatment will initiate a new follicular wave due to dominant follicle luteinization, or by chance, is still controversial. The likelihood of ovulation would seem to depend on follicular wave status at the time of GnRH treatment. If ovulation is a prerequisite for the development of a new follicular wave, then the success of GnRH-based protocols is dependent on stage of the follicle development when treatment is initiated. On the contrary, estradiol/progestin protocols may be a better alternative than GnRH-based protocols when treatment is initiated at random stages of the estrous cycle, as these two steroids are potentially able to induce atresia of both FSH- and LH-dependent follicles. In Chapter 6, 100 μg GnRH or 1 mg ECP was used to synchronize follicular wave emergence. Although we did not conduct daily ultrasonographic examinations to compare efficacy of treatment on ovarian follicular dynamics, pregnancy rate following TAI (56.1 and 56.4% for GnRH and ECP treatments, respectively) indirectly indicate that treatments were equally efficacious in synchronizing dominant (ovulatory) follicle development. Treatment with 1 mg ECP in Chapter 5 (Experiment 2) resulted in 57% of heifers with synchronous follicular wave emergence 3 to 4 d later. Other researchers have reported that only 54 to 56% of heifers ovulated following treatment with GnRH at random (Pursley et al., 1995), or at predetermined (Martínez et al., 1999) stages of the estrous cycle. Interestingly, the proportion of heifers that responded to the injection of GnRH by ovulating a dominant follicle (Pursley at al., 1995; Martínez et al., 1999) was similar to proportion of heifers that responded to the injection of ECP by initiating synchronous emergence of a new follicular wave (Chapter 5, Experiment 2).

As stated earlier, follicular wave emergence must be induced in progestin-based estrus synchronization protocols to avoid the development of persistent follicles. Most progestin-treated-cattle that failed to respond to the treatment given to induce a new follicular wave developed a persistent follicle, which has been shown to result in reduced conception rates after AI. As it turns out, most of the nonresponding cattle were at late stages of the estrous cycle, or in proestrus, when treatment was administered. Martínez et al. (2002a) reported that estradiol/progesterone treatment did not consistently induce emergence of a new follicular wave in heifers undergoing spontaneous luteal regression at the time of treatment. Bó et al. (1994) also reported that estradiol treatment more effectively suppressed growth of the first-wave dominant follicle when administered with a progestogen implant than when administered alone in heifers during metestrus, indicating that the efficacy of estradiol treatment depends on circulating progesterone concentrations. On the possibility of encountering cows without a functional CL in a group of randomly cycling cows, we have consistently included progesterone with the first estrogen treatment. Even though treatment with a combination of estradiol and progesterone at the time of CIDR insertion may not consistently induce regression of the dominant follicle in cattle with low circulating progesterone, the progesterone may have a positive effect on fertility. Consequently, in studies reported in Chapter 8, we hypothesized that the ability of estradiol to consistently induce follicle atresia and synchronize follicular wave emergence is dependent on elevated plasma progesterone concentrations. Martinez et al. (2003a) have shown that new CIDR inserts in ovariectomized beef cows increased plasma progesterone concentrations by approximately 7 ng/mL within 24 h. However, if a once-used CIDR was inserted, an

injection of 100 mg of progesterone was needed to increase plasma progesterone concentrations an equivalent amount. We speculated that the magnitude of the sudden increase in circulating progesterone concentrations within 24 h of CIDR insertion would affect the ability of estradiol to induce atresia followed by synchronous follicle development. However, pregnancy rate following TAI did not differ between cattle receiving a new versus a once-used CIDR insert, whether or not progesterone was administered at the time of CIDR insertion. Our hypothesis was rejected, and we speculated that the 2-3 ng/mL increases in plasma progesterone concentrations in cattle treated with a once-used CIDR (Martínez et al., 2003a) was sufficient to achieve acceptable pregnancy rates following TAI. In contrast, cattle treated with a twice-used CIDR had the lowest pregnancy rate following TAI. A twice-used CIDR may have increased circulating progesterone concentrations insufficiently for estradiol treatment to induce follicle atresia because an injection of 100 mg progesterone tended to increase pregnancy rate in animals receiving a twice-used CIDR. However, a twice-used CIDR may have also not maintained plasma progesterone concentrations sufficiently to prevent an LH surge during the treatment period.

All new CIDR inserts used in the studies reported in this thesis had 1.9 g of progesterone. Macmillan et al. (1991) reported that ovariectomized beef heifers treated with new CIDR for 12 d had average plasma progesterone concentrations of 5.6 ng/mL during treatment, varying from 8.7 ng/mL within 6 h of insertion to 2.5 ng/mL at CIDR removal. Similarly, treatment of ovariectomized dairy cows with a CIDR insert increased plasma progesterone concentrations from 0.3 to 8.6 ng/mL within 6 h, followed by a

decline to 2.3 ng/mL at CIDR removal (Peterson and Henderson, 1991). Based on these data, CIDR inserts used for 7 (once-used) or 14 d (twice-used) should be able to maintain plasma progesterone concentrations above 1.5 ng/mL, which should suppress an endogenous LH surge and ovulation. However, methods of cleaning, disinfecting, and storage may have reduced progesterone content in some previously used CIDR inserts utilized in our studies. Macmillan and Peterson (1993) reported that the average progesterone content in 1.9 g CIDR inserts previously used for 15 d was 0.92 g. The amount of progesterone remaining in a used CIDR after 15 d insertion period is primarily dictated by the initial progesterone content and was not affected by inserting more than one insert (Macmillan et al., 1990). In this study, the average residual progesterone content of the used inserts was the same (1.07 g) irrespective of whether they were from cows treated with one or three CIDR inserts, indicating that the progesterone absorption was not affected by the number of CIDR inserted. We also expected that two twice-used CIDR inserts would increase plasma progesterone concentrations in beef heifers, but pregnancy rate was similar in heifers treated with two twice-used CIDR inserts to that in those receiving only one twice-used CIDR insert.

Treatments for synchronizing follicular wave emergence that are reported in this thesis are summarized in Table 11.1. It is clear that administration of 5 mg E-17β at progestin treatment consistenly resulted in emergence of a new follicular wave regardless of the progestin used and whether or not progesterone was injected at the same time. The mean (± SD) interval from treatment to follicular wave emergence varied from 3.0 ± 1.1 to 3.6 ± 0.5 d. The same may be said of 1 or 2 mg EV with follicle wave emergence

occurring 3.2 ± 0.9 and 3.4 ± 0.8 d after treatment, respectively. Conversely, the inteval from treatment with 5 mg of EV to follicular wave emergence was longer and more variable (5.7 ± 1.5 and 4.8 ± 1.2 d in heifers and cows, respectively). Similarly, administration of 1 mg of ECP, another estradiol ester, resulted in considerable variability in the interval from treatment to follicular wave emergence (standard deviations varied from 1.4 to 4.1 d in heifers and cows, respectively). Hence, one could question whether follicle wave emergence was any more synchronous following administration of ECP (with or without progesterone) than the administration of a CIDR insert alone or plus 150 mg of progesterone, which we concluded did not synchronize follicular wave emergence (standard deviations ranged from 2 to 3.7 d). Treatment with 100 μg GnRH resulted in follicular wave emergence in 0.8 ± 0.8 d in "presynchronized" cows in studies reported in Chapter 10. However, caution must be used in interpreting this result because animals at random stages of the estrous cycle were not studied. As indicated above, it has been previously shown that presynchronization procedures improve the efficacy of GnRH-based TAI protocols by providing for an LH-responsive follicle at the time of the first GnRH treatment.

Table 11.1. Mean (+ SD) day of follicular wave emergence (FWE) following treatment at CIDR or Norgestomet implant insertion (all experiments combined).

Experiment (Chapter)	Treatment	Estrous cycle (d)	Animal	n	FWE (Mean ± SD)	FWE Range
3(7)	nCIDR	at random	cows	10	3.8 ± 2.0	(-4 to 6)
1(10)	oCIDR	12 ± 0.7	cows	10	0.2 ± 3.7	(-5 to 6)
1(10)	oCIDR+150mg P4	12 ± 0.7	cows	11	-0.2 ± 3.3	(-6 to 5)
3(5)	nCIDR+5mg E-17β	at random	heifers	15	3.0 ± 1.1	(-6 to 4)
3(5)	nCIDR+5mg E-17β+100mg P4	at random	heifers	15	3.4 ± 0.9	(-5 to 5)
2(5)	oCIDR+5mg E-17β+100mg P4	at random	heifers	30	3.3 ± 0.8	(2 to 5)
1(7)	Norgestomet(I)+5mg E-17β+100mg P4	at random	heifers	35	3.6 ± 0.5	(3 to 4)
1(5)	oCIDR+1mg ECP	at random	heifers	13	3.7 ± 1.8	(2 to 7)
1(10)	oCIDR+1mg ECP	12 ± 0.7	cows	11	0.7 ± 4.1	(-6 to 5)
1(5)	oCIDR+1mg ECP+50mg P4	at random	heifers	11	4.0 ± 1.6	(2 to 7)
2(5)	oCIDR+1mg ECP+100mg P4	at random	heifers	28	4.1 ± 1.4	(-4 to 8)
3(7)	nCIDR+1mg EV	at random	cows	11	3.2 ± 0.9	(-4 to 4)
3(7)	nCIDR+2mg EV	at random	cows	10	3.4 ± 0.8	(2 to 5)
3(7)	nCIDR+5mg EV	at random	cows	11	4.8 ± 1.2	(3 to 7)
1(7)	Norgestomet(I)+5mg EV+3mg Norgestomet	at random	heifers	38	5.7 ± 1.5	(3 to 9)
1(10)	oCIDR+100μg GnRH	12 ± 0.7	cows	11	0.8 ± 0.8	(-1 to 2)

oCIDR = Once-used CIDR; nCIDR = New CIDR; Norgestomet(I) = Norgestomet implant
E-17β = Estradiol-17β; ECP = Estradiol cypionate; EV = Estradiol valerate; P4 = Progesterone.

11.2 Synchronizing follicular wave emergence for superovulation treatments

The objective of ovarian superstimulatory treatment with exogenous gonadotrophins in cattle is to obtain the maximum number of viable embryos by stimulating growth and subsequent ovulation of competent antral follicles. Follicular response to superstimulation treatments remains variable both within and between cattle. The unpredictability of response causes severe logistical problems and contributes to the high cost of embryo production in conventional embryo transfer programs. However, superovulatory response of cattle is affected by various factors. It is well known that the presence of a dominant follicle at the time of gonadotrophin treatments will reduce the number of transferable embryos (Guilbault et al., 1991; Bungartz and Niemann, 1994). The synchronization of follicular wave emergence not only avoids the presence of a dominant follicle at initiation of gonadotrophin treatment, but it also allows for the scheduling of embryo collection and transfer at the most convenient time from a management perspective. Previous studies have shown the importance of synchrony between follicular wave emergence and initiation of gonadotrophin treatments (Nasser et al., 1993; Baracaldo et al., 2000). As little as one day of asynchrony resulted in decreased numbers of transferable embryos (Nasser et al., 1993). Therefore, we hypothesized that the estradiol/progesterone treatments that result in the most synchrony of follicular wave emergence would result in a higher superovulatory response in term of numbers of transferable embryos (Chapter 7). In this regard, E-17β treatment has consistently resulted in a greater degree of synchrony of follicular wave emergence, and FSH treatment 4 d later has tended to result in a consistently high number of transferable

embryos. However, the most important benefit is that procedures can be done at a practitioner-appointed time, without consideration of the stage of the estrous cycle.

11.3 Synchronizing LH surge and ovulation

The use of exogenous estradiol as part of an estrus synchronization protocol is also based on the ability of estradiol to induce an LH surge by stimulating hypothalamic secretion of GnRH (Nett et al., 1984; Turzillo and Nett, 1999). Estradiol also increases pituitary sensitivity to GnRH, apparently by increasing the number of GnRH receptors within the gonadotrope cells (Turzillo et al., 1994).

In Chapter 5, we hypothesized that a small dose of ECP would synchronize LH release and ovulation in heifers in which circulating concentrations of progesterone had been caused to decline. It was also hypothesized that due to the prolonged elevation of estradiol in the circulation, administration of ECP at CIDR removal would result in acceptable pregnancy rates following TAI. The first hypothesis was supported by studies presented here. The administration of 0.5 mg ECP 24 h after CIDR removal synchronized LH release and ovulation in beef heifers. The second hypothesis was partially supported. In this regard, an interesting observation was made in Experiment 2 (Chapter 5) and confirmed later in larger studies in Experiment 4, and in Chapter 6. Pregnancy rate following TAI in heifers treated with ECP at CIDR removal depended on the treatment used to synchronize follicular wave development. When GnRH or ECP had been given to synchronize follicular wave emergence, pregnancy rate was higher in heifers treated with

ECP 24 h after CIDR removal. Conversely, if E-17β had been administered at CIDR insertion to synchronize follicle wave emergence, then the timing of ECP treatment to induce LH release and ovulation was less critical. The administration of ECP at CIDR removal resulted in pregnancy rates following TAI that did not differ from that when ECP was administered 24 h later. The reduced fertility observed following ECP treatment at CIDR removal in animals that had received ECP or GnRH to synchronize follicular wave emergence was likely due to asynchronous ovulation following asynchronous emergence of the ovulatory follicle. The administration of E-17β resulted in much more synchronous emergence of a growing dominant follicle than either GnRH or ECP. Several precocious ovulations occurred in ECP- and GnRH-treated heifers that received ECP at CIDR removal in Experiment 2 (Chapter 5). Overall, heifers that ovulated before TAI may have had low progesterone concentrations; estradiol treatment or an estrogen-active dominant follicle may have induced an early LH surge, resulting in premature ovulation.

That ECP administered at CIDR removal resulted in acceptable pregnancy rates in E-17β-treated cattle is a very important finding, particularly for cow-calf operations. In extensively managed cattle herds, it is necessary to sort cows from calves every time that treatments must be administered. Increased animal handling is not only stressful to both cows and calves, but also requires input in time and labor for producers. Hence, estrus synchronization protocols that reduce the number of handling procedures would be well accepted among beef cattle producers.

Although we did not investigate the effect of dose of ECP in beef cows, Pancarci et al, (2002) have suggested that 1 mg ECP is more efficacious than 0.5 mg ECP in synchronizing ovulation in lactating Holstein cows. However, the author of this thesis has utilized the CIDR-ECP protocol in lactating beef cows with acceptable pregnancy rate following TAI (unpublished). Wiltbank et al. (2000) have reported that lactating dairy cows had increased steroid hormone metabolism, which may have affected the effectiveness of 0.5 mg ECP in the study by Pancarci et al. (2002).

GnRH has been used to synchronize the LH surge and ovulation in estrus synchronization and TAI schemes. Geary et al. (2001) demonstrated that GnRH could be given at TAI without a detrimental effect on pregnancy rate when compared to GnRH given 16 h before TAI. In our study, pregnancy rate following TAI in heifers (Chapter 6) was not only affected by timing of ECP treatment, but also by type of treatment given after CIDR removal. Administration of ECP 24 h after CIDR removal resulted in higher pregnancy rates than GnRH treatment at the time of TAI, whether ECP or GnRH was administered at the time of CIDR insertion. A difference in CL formation and function may occur in cattle that have a spontaneous or estradiol-induced LH surge versus a GnRH-induced LH surge. The spontaneous (Thatcher and Chenault, 1976) or estradiol-induced (Bó et al., 1994; Martínez et al., 2003a) preovulatory LH surge is of a longer duration (approximately 10 h) as compared to that following an injection of 100 μg GnRH (approximately 4 h; Chenault et al., 1990; Martínez et al., 2003b).

The optimal duration and amplitude of the LH surge required for ovulatory events has been studied extensively in humans and primates; in both, the LH surge of spontaneous cycles had a duration of 48-50 h (Weick et al., 1973; Hoff et al., 1983). Endogenous LH surges of 14 h or less duration elicited by GnRH or GnRH agonist treatment were insufficient to induce periovulatory events in oocytes and granulosa cells in primates (Zelinski-Wooten et al., 1991; Chandrasekher et al., 1991). However, an LH surge of up to 24 h in primates induced oocyte maturation and granulosa cell luteinization, but did not support CL function (Zelinski-Wooten et al., 1992). Longer LH surges, (up to 48 h) similar to those endogenous surges, are needed to promote CL development and function in humans (Lanzone et al., 1989) and primates (Chandrasekher et al., 1994). Thus, genes involved in luteinization (e.g. steroidogenic enzymes StAR and $P450_{scc}$) are also induced by the LH surge. The extent of the LH surge may have an effect on expression of genes necessary for steroid production by the new CL. A more-developed and metabolically active CL may result in higher progesterone concentrations during the luteal phase. Recent studies in cattle suggest that many of the ovulations resulting from GnRH treatment may not result in normal CL formation. Cordoba and Fricke (2002) reported that about 12% of cows had a second insemination within 18 d following an Ovsynch® protocol in which a dose of 50 μg GnRH was used. Short cycles were also observed following an Ovsynch® protocol in which a dose of 100 μg GnRH was used (Shephard, 2002; Colazo unpublished). Abnormal CL formation seems not to be related to the dose of GnRH because differences in CL development and progesterone production were also found between cows with spontaneous or induced ovulation following administration of 250 μg of gonadorelin (Macmillan et al., 2003).

There is also evidence that inadequate estradiol secretion by the preovulatory follicle may cause failure of normal luteal function or premature secretion of luteolytic PGF. Benoit et al. (1992) observed a delay in initiation of luteal function when ewes were treated with an aromatase inhibitor and then treated with hCG to induce ovulation in the absence of an estrogen-induced LH surge. A number of authors have reported sub-optimal levels of preovulatory estradiol secretion preceding the formation of CL with short-life span (Garcia-Winder et al., 1986; Garverick et al., 1988). Inadequate preovulatory estradiol production leads to inadequate inhibition of oxytocin receptors in the uterus, resulting in premature secretion of PGF (Mann and Laming, 2000). Premature uterine secretion of PGF and a short luteal phase following first ovulation following onset of puberty, or post-partum or seasonal anestrus is common in ruminants. Pretreatment with progesterone has usually resulted in formation of a CL with a normal functional lifespan (Ramirez Godinez et al., 1981). However, prevention of the early secretion of PGF induced by oxytocin in ovariectomized cows required a sequence of exposure to estrogen and progesterone preceded by progesterone priming (Kierborz-Loos et al., 2003).

Although estrus detection was not performed in the study described in Chapter 6, fewer GnRH-treated heifers were expected to show behavioural estrus than ECP-treated heifers. Estrus detection, and indeed, whether or not estrus is expressed, is not important if ovulation is synchronized for TAI. Nevertheless, estrus rate before TAI was higher in heifers treated with estradiol (92.0%) than in those treated with GnRH (50.7%) or pLH (47.2%) to induce ovulation (Martinez et al., 2002c). Interestingly, pregnancy rates were

higher in GnRH- or pLH-treated heifers that were detected in estrus, but pregnancy rates were not different in estradiol-treated heifers that did or did not show behavioural estrus.

The use of long acting estradiol esters, such as ECP, to synchronize ovulation may be of some concern due to elevated concentrations of estradiol during ovum transport and fertilization, and early embryonic and CL development. However, results presented here did not support that concern. Conversely, estradiol has been shown to facilitate sperm transport in the female reproductive tract (Harper, 1994). Treatment with estradiol during proestrus increased the number of sperm in the oviducts, uterus and cervix after natural mating (Haw and Cooper, 1975). In cattle and sheep, an oviduct-specific glycoprotein secreted mainly from Days -1 to 3 of the estrous cycle is synthesized by the ampulla region of the oviduct in response to estrogen (Nancarrow and Hill, 1995). This protein, and other amino acids, may interact with the gametes or embryo to facilitate the processes of fertilization and development. Therefore, the beneficial effects of estradiol during fertilization and embryo development may lead to increased pregnancy rates when ECP, rather than GnRH, is used to synchronize ovulation.

Pregnancy rates following TAI for all experiments combined are shown in Table 11.2. Treatment protocols in these studies differed somewhat, but they were all designed to synchronize follicle wave emergence and ovulation so as to facilitate TAI. Although group sizes varied considerably, pregnancy rates ranged from 44.3 to 100 % in 2507 cattle. As discussed in Section 11.5, many other factors may have affected fertility following TAI, and nutrition and management are probably two of the most important.

Therefore, it is not the intention to draw conclusions from this table, but rather to present a summary of pregnancy rates following the use of similar or dissimilar protocols, all designed to accomplish the same thing. For example, data presented in Chapter 10 suggest that GnRH was quite efficacious in synchronizing follicle wave emergence while ECP was not (see Table 11.1). However, ECP was at least as efficacious as GnRH (without presynchronization) in synchronizing follicular wave emergence and ovulation for TAI in Chapter 6 (see Table 11.2). The greatest benefit for the use of ECP seemed to be in the synchronization of LH release and ovulation. As one might expect, the synchrony of ovulation may be more critical than the synchrony of follicle wave emergence for the successful application of TAI. In other words, protocols for TAI would appear to have considerable flexibility in the synchronization of follicle wave emergence, providing the timing of ovulation was well controlled and AI was conducted at the appropriate time.

Table 11.2. Pregnancy rates (%) following timed-AI (TAI) for all experiments combined. Heifers received a CIDR insert for 7 or 9 d (brackets) and prostaglandin was administered at CIDR removal. TAI was done relative to CIDR removal.

Experiment (Chapter)	Treatment (d)	TAI (h)	Animal	n	Preg. rate (%)
2(5)	oCIDR(7)+5mg E-17β+100mg P4+0.5mg ECP0	58-60	heifers	10	70
2(5)	oCIDR(7)+5mg E-17β+100mg P4+0.5mg ECP24	58-60	heifers	10	60
2(5)	oCIDR(7)+5mg E-17β+100mg P4+1mg EB24	58-60	heifers	10	80
4(5)	nCIDR(7)+5mg E-17β+100mg P4+0.5mg ECP0	54-56	heifers	98	63.3
4(5)	nCIDR(7)+5mg E-17β+100mg P4+0.5mg ECP24	56-58	heifers	99	64.6
4(5)	nCIDR(7)+5mg E-17β+100mg P4+0.5mg EB24	56-58	heifers	103	63.1
2(8)	oCIDR(7)+1mg EB+100mg P4+1mg EB24	52-56	heifers&cows	93	64.5
2(8)	oCIDR+1mg EB+1mg EB24	52-56	heifers&cows	88	60.2
2(8)	tCIDR(7)+1mg EB+1mg EB24	52-56	heifers&cows	92	45.6
2(8)	tCIDR(7)+1mg EB+100mg P4+1mg EB24	52-56	heifers&cows	90	51.1
3(8)	nCIDR(7)+2mg EB+50mg P4+1mg EB24	52-56	heifers	33	57.5
3(8)	oCIDR(7)+2mg EB+50mg P4+1mg EB24	52-56	heifers	58	63.8
3(8)	tCIDR(7)+2mg EB+50mg P4+1mg EB24	52-56	heifers	48	47.9
3(8)	2tCIDR(7)+2mg EB+50mg P4+1mg EB24	52-56	heifers	48	47.9
1(8)	nCIDR(9)+1mg ECP+0.5mg ECP24	55-60	heifers	151	49.6
1(8)	oCIDR(9)+1mg ECP+0.5mg ECP24	55-60	heifers	156	48.0
2(5)	oCIDR(9)+1mg ECP+100mg P4+0.5mg ECP0	58-60	heifers	9	44.4
2(5)	oCIDR(9)+1mg ECP+100mg P4+0.5mg ECP24	58-60	heifers	10	100
2(5)	oCIDR(9)+1mg ECP+100mg P4+1mg EB24	58-60	heifers	9	55.5
1(6)	oCIDR(9)+1mg ECP+50mg P4+0.5mg ECP0	58-60	heifers	160	56.2
1(6)	oCIDR(9)+1mg ECP+50mg P4+0.5mg ECP24	58-60	heifers	165	64.8
1(6)	oCIDR(9)+1mg ECP+50mg P4+100μg GnRH52	52-54	heifers	166	48.2
1(8)	nCIDR(9)+1mg ECP+100mg P4+0.5mg ECP24	55-60	heifers	149	46.9
1(8)	oCIDR(9)+1mg ECP+100mg P4+0.5mg ECP24	55-60	heifers	160	44.3
1(6)	oCIDR(8)+100μg GnRH+100μg GnRH52	52-54	heifers	165	53.9
1(6)	oCIDR(8)+100μg GnRH+0.5mg ECP0	58-60	heifers	162	48.1
1(6)	oCIDR(8)+100μg GnRH+0.5mg ECP24	58-60	heifers	165	66.1

oCIDR = Once-used CIDR; nCIDR = New CIDR; tCIDR = Twice-used CIDR

E-17β = Estradiol-17β; ECP = Estradiol cypionate; EV = Estradiol valerate; EB = Estradiol benzoate; P4 = Progesterone.

ECP0 = ECP treatment at CIDR removal; ECP24 = ECP treatment 24 h after CIDR removal; EB24 = EB treatment 24 h after CIDR removal; GnRH52 = GnRH treatment 52 h after CIDR removal.

11.4 Time of PGF treatment and progestin withdrawal

During proestrus (low progesterone environment), pulses of LH measured in the plasma occur with a high frequency and low amplitude (Rahe et al., 1980). LH pulse frequency increases as estradiol concentrations increase, leading to the preovulatory LH surge (Chenault et al., 1975). Hence, estradiol treatment would increase pituitary sensitivity to GnRH release and contribute to these episodes of increased LH release, which probably up-regulate mechanisms that contribute to final stages of follicular development. Furthermore, the preovulatory follicle expresses steroidogenic enzymes necessary for the synthesis of estradiol, which in turns triggers the LH surge. The preovulatory LH surge then acts on granulosa and theca cells to terminate gene expression associated with folliculogenesis (the CL of humans, pigs and rats retain the ability to produce estradiol) and this change in gene expression precedes luteinization or ovulation of the dominant follicle (Niswender et al., 2000).

In estrus synchronization protocols, luteolysis is usually induced shortly after the establishment of dominance by the induced-ovulatory follicle. For example, in GnRH- (e.g. Ovsynch® and Cosynch®) or estradiol/progestin-based protocols, PGF is given 7 d after the first GnRH or estradiol treatment. A new dominant follicle should emerge, on average, 2 and 4 d after GnRH and estradiol treatment, respectively, in cattle responding to the initial treatment. Therefore, when luteolysis is induced, GnRH-treated cattle will have a dominant follicle that is 2 d older and larger than estradiol-treated cattle. Interestingly, ovulatory follicles are approximately 3 d older and 2 mm larger at the time of ovulation in two-wave than in three-wave cattle (Ginther et al., 1989b; Townson et al.,

2002; Bleach et al., 2004). Conception rate to AI has been reported to be lower in lactating dairy cows with two (63%), rather than three (81%) waves of follicular development (Townson et al., 2002). However, Bleach et al. (2003) report no diference in fertility between 2- and 3-wave dairy cows. The ovulatory wave emerges on Days 10 or 16 and luteolysis occurs on Day 16.5 or 19 in two and three wave animals, respectively (Ginther et al., 1989b). Hence, the interval from ovulatory follicle emergence to luteal regression is approximately 3 d longer in 2-wave animals. Therefore, the age and larger size of the ovulatory follicle in two-wave animals is due to the period before luteolysis, in the same way as would occur in cattle treated with GnRH-based protocols. If there is a difference in fertily between 2- and 3-wave cattle, it would have to be a result of the interval from follicular wave emergence to luteolysis, and not due to the interval from luteolysis to ovulation.. However, the effect of the length of proestrus on fertility in TAI protocols still remains unresolved.

A retrospective analysis of ovulatory follicle diameter between open and pregnant heifers in Experiment 2 (Chapter 5) suggested that an important factor related to fertility was size of the preovulatory follicle. Heifers that did not conceive had a smaller preovulatory follicle (12.7 ± 1.3 mm diameter) at the time of TAI than those that became pregnant (13.6 ± 1.6 mm). Recent studies, in which GnRH was used to induce ovulation, clearly showed that fertility is compromised when animals are induced to ovulate a dominant follicle that has not reached full maturity (Vasconcelos et al., 2001; Mussard et al., 2003; Colazo et al., 2004c). In essence, a small ovulatory follicle resulted in low pregnancy rates following TAI. In a study in which follicle wave emergence was induced by follicle ablation, mean ovulatory follicle diameter and conception rate was higher in

cattle with spontaneous ovulation (12.0 ± 0.3 mm and 100%) than those in which GnRH was administered when the largest follicle was 10 mm in diameter (10.7 ± 0.1 mm and 75.9%; Mussard et al., 2003). When PGF was given approximately 4.5 d after follicle ablation, conception rates were 4.4 and 57.4 % in cattle induced to ovulate follicles of 11.1 or 13.6 mm in diameter, respectively. Mussard et al. (2003) suggested that follicle size was not the only component affecting fertility; length of proestrus and the associated endocrine environment to which a follicle has been exposed may also affect fertility. Exposure to increased LH pulse frequency and elevated estradiol concentrations characteristic of proestrus may also affect oocyte maturation and fertility. In this regard, there is evidence of an influence of follicular fluid (especially estradiol concentrations in follicular fluid) from different follicle populations, on oocyte maturation and blastocyst production in vitro (Sirard et al., 1995). The proportion of embryos reaching the 64-cell stage and the average number of nuclei per embryo at the 16- and 32-cell stages, were higher for oocytes maturated in the presence of follicular fluid from a dominant follicle than those matured in the presence of follicular fluid from superovulated animals (Sirard et al., 1995).

It has been also shown that larger preovulatory dominant follicles form larger CLs that produce greater amounts of progesterone (Vasconcelos et al., 2001; Burke et al., 2001; Mussard et al., 2003), resulting in lower embryonic mortality (Perry et al., 2003) and higher pregnancy rates following AI (Vasconcelos et al., 2001; Mussard et al., 2003; Colazo et al., 2004). Cattle induced to ovulate immature follicles had CL that produced plasma progesterone concentrations that were 61 to 89% that of normal CL (Mussard et

al., 2003). Ewes induced to ovulate 24 h early also had subfunctional CLs that were deficient in large luteal cells (Murdoch and Van Kirk, 1998), and had attenuated endometrial gland development and gestational failure. Increased progesterone concentrations have been shown to be also associated with increased conceptus size and an increased ability to produce interferon tau (INF-τ; Mann et al., 1999), which is necessary for maternal recognition of pregnancy.

Fertility would appear to be affected for several mechanisms. It has been suggested that extending the length of progestin treatment by 1d will result in a more synchronized ovulation (Macmillan et al., 1999), larger ovulatory follicles and a subsequent increase in pregnancy rate following TAI (Bó, personal communication 2003). However, we were unable to show any difference in pregnancy rates following TAI in beef cattle treated with estradiol and a CIDR insert for either 7 or 8 d (unpublished). A longer progestin treatment may also result in a shorter interval from CIDR removal and PGF treatment to ovulation.. Perhaps size of the ovulatory follicle is not exclusively important and subtle changes in the endocrine environment in which the ovulatory follicle develops may affect oocyte competence, CL function and subsequently embryo development.

In Chapter 7, estradiol valerate (EV)-treated heifers had a shorter interval from follicular wave emergence to ovulation than E-17β- treated heifers. Although follicular wave emergence was more synchronous and dominant follicle size at norgestomet implant removal was larger in E-17β-treated heifers, the interval to ovulation was more

synchronous in heifers treated with 5 mg EV. The EV treatment presumably caused luteal regression prior to progestin implant removal, while E-17β probably did not, so that when the norgestomet implant was removed, progestin concentrations fell more rapidly, and the suppressive effects of progesterone on LH release was removed earlier in EV-treated animals. In addition, the prolonged circulating blood levels of estradiol following the administration of 5 mg EV may have stimulated behavioural estrus and induced LH release shortly after norgestomet implant removal (Larson and Kiracofe, 1995; Martínez et al., 2003a). As synchrony of ovulation is required to achieve high pregnancy rates following TAI, cattle with synchronized follicular development and reduced concentrations of circulating endogenous progesterone at the time of progestin removal may have had more synchronous LH release and ovulation. Although the effects of low progestin concentrations on follicle growth and oocyte development have not been thoroughly studied, the subsequent high fertility warrants further investigation.

11.5 Other factors that may affect fertility

It appears that the timing of AI is a compromise, where success in inseminating early appears to be limited by sperm life leading to fertilization failure and inseminating late is limited by declining oocyte viability and embryonic quality (Saacke et al., 2000). Using the HeatWatch™ system to identify onset of estrus, inseminations were performed approximately at 2, 12, or 24 hours following the first mount (Dalton et al., 2001) and embryos were recovered non-surgically 6 d later. When inseminations were delayed, fertilization rate and median number of accessory sperm increased from approximately

66 to 82% and from 0 to 4/ovum, respectively. In contrast, embryo quality declined (78 to 45%) for inseminations at onset of estrus to those performed 12 or 24 h later. Results indicate that AI 12 h after the onset of estrus provides a preferred compromise between potential fertilization failure and embryo failure, resulting in higher overall fertility. The longer the interval between AI and ovulation, the higher the embryo quality, suggesting that length of sperm residence in the female tract provides additional selection for more competent sperm. In cattle synchronized with estradiol/progesterone schemes, it is recommended that TAI be performed 48 to 52 h after CIDR removal (or 24 to 28 h after estradiol treatment). Based on endocrine and ultrasonographic studies conducted in our lab, this recommendation needs to be adjusted. Although timing of AI will mainly depend on hormone preparation used to synchronize ovulation, preliminary data indicate that following the use of a CIDR/EB protocol, TAI at 33 to 36 h yields higher pregnancy rates than TAI at 24 h (Whittaker et al., 2002; Colazo et al., 2003a). However, this must be confirmed in more comprensive studies.

Accessory sperm trapped in the zona pelucida are believed to reflect the number of spermatozoa competing for fertilization (Saacke et al., 1998) and be associated with fertility (Hunter and Wilmut, 1984). In addition, the number of accessory sperm and fertility have been shown to increase with increasing dosages of either frozen or fresh spermatozoa (Nadir et al., 1993). Dalton et al. (2001) also compared fertilization rate and accessory sperm per ovum between AI and natural service. Fertilization rate and accessory sperm per ovum differed between AI at 12 h after onset of estrus and natural service (79 versus 98% and 10 versus 27 sperm/ovum, respectively). Embryo quality was

numerically higher after natural service than AI (approximately 78 versus 61%). Although differences in ejaculate volume and sperm survival (fresh versus frozen) may explain the higher fertility following natural service, sperm selection may have potentially increased the population of more competent sperm. The authors suggested that potential benefits would come from more research designed to optimize sperm preservation technology or identification of biologically important components of fresh semen that would favor the functional life of sperm in vivo. It is important to note that in Dalton's study, bull variation in accessory sperm number was large (median number of accessory sperm per ovum for the three bulls was 73, 37 and 2), even though 3 bulls considered to be fertile were used for natural service.

Sire variation in fertility may be further magnified when frozen-thawed semen is used for TAI. Records for 6012 inseminations of cows were analyzed to determine the effect of the stage of estrus at the time of inseminations on conception rates for 3 groups of fertile sires (Macmillan and Watson, 1975). When cows were inseminated at the end of estrus, average conception rates were 73.3% for 7 sires of above average fertility, 71.3% for 5 sires of average fertility, and 68.3% for 6 sires of below average fertility. In contrast, when cows were inseminated at onset of estrus, conception rates were 74.3, 62.7, and 58.4% for the 3 groups of sires, respectively. These differences may be exacerbated with TAI, and may not be explainable by conventional sperm viability or morphological assessment assays. Hence, new approaches to the examination of bull fertility for TAI are needed.

Recently, antiluteolytic strategies to improve fertility in cattle have been reviewed (Binelli et al., 2001). Strategies include hormonal and nutritional manipulations to decrease plasma concentrations of estradiol while increasing plasma concentrations of progesterone, and inhibiting the PGF-synthesizing enzymatic machinery in the endometrium during maternal recognition of pregnancy. We attempted to improve fertility in heifers submitted to TAI by nutritional manipulation to inhibit the PGF-synthesizing enzymatic machinery (Chapter 6). Prostaglandin F-2α is synthesized mainly from arachidonic acid (omega-6 fatty acid). Thatcher et al. (1994) identified linoleic acid (omega-6 fatty acid) as an endometrial PGF synthetase inhibitor. Two other lipids with cyclooxygenase-2 (COX-2; enzyme that catalyze the conversion of arachidonic acid to PGF) inhibiting activity are eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids (Levine and Worth, 1984). Although, most polyunsaturated fatty acids (PUFA) are preferentially incorporated in the metabolic pathway of arachidonic acid, linolenic acid (omega-3 fatty acid) also leads to EPA synthesis (Bereziat, 1978). Flax seed is a rich source of linolenic acid and sunflower seed is rich in linoleic acid (Ambrose and Kastelic, 2003). It was hypothesized that feeding these two oilseeds would improve pregnancy rates in heifers submitted to estrus synchronization and TAI. Results of the experiment described in Chapter 6 did not support this hypothesis. There was no significant effect of oilseeds on fertility. In contrast, other studies reported increased fertility in lactating dairy cows following the addition of either linoleic or linolenic acid to the diet (Staples et al., 1998; Petit et al., 2001; Ambrose et al., 2002). There may be important differences between these studies and our study. Luteolytic mechanism in lactating dairy cows may be more sensitive than beef heifers to changes in fatty acids in the diet. As previously

mentioned, processing the oilseeds may have resulted in higher pregnancy rates, or an increase in pregnancy rates may be more likely when fertility is low to moderate in the control group. We numerically improved pregnancy rate by feeding oilseed, but the difference was not statistically significant. The 5% improvement in pregnancy rate was mainly in heifers treated with GnRH at TAI or ECP at CIDR removal. Both treatments resulted in a statistically lower pregnancy rate than ECP treatment 24 h after CIDR removal. Therefore, supplementation of oilseeds in the diet is most likely to increase pregnancy rate when other factors are limiting fertility.

Poor nutritional management of cattle involved in estrus synchronization for TAI has been shown to negatively affect fertility. Negative energy balance clearly affects a variety of circulating hormones such as growth hormone, insulin like growth factor-I (IGF-I), insulin, leptin, cortisol or thyroxine, all of which have been shown to affect bovine follicular cell proliferation, or steroidogenesis in vitro (reviewed in Mihm and Bleach, 2003). These metabolic hormones may exert their effects on follicles or oocytes through gonadotrophin release or directly on the ovary. It is remarkable that FSH-dependent follicle growth and dominant follicle selection were not perturbed even under severe chronic undernutrition (Rhodes et al., 1995). However, an acute nutritional deficit influenced growth and estradiol synthesis of preovulatory follicle (Mackey et al., 1999). Although these two alterations were more likely to be mediated by insulin or IGF-I, others metabolic hormones such as leptin should not be ruled out (Kendall et al., 2004). Increased conception rates have been observed following the feeding of a diet which results in increased circulating concentrations of insulin in postpartum dairy cows (Gong

et al., 2002), suggesting that metabolic hormones may alter oocyte competence. Other important factors that affect fertility in cattle synchronized for TAI are body condition score, cyclicity, and body weight in heifers and days post partum in cows (Roche et al., 1978; reviewed in Rhodes et al., 2003).

11.6 Resynchronization studies

The rationale for insertion of a previously used CIDR, or of feeding MGA to previous TAI animals, was to delay estrus in animals that were not pregnant following TAI or may have had early luteolysis. Progestin treatment (starting 13 ± 1 d after TAI) synchronized returns to estrus. Compared to untreated control heifers, the range interval to estrus following TAI was reduced by 6 and 9 d (see Experiments 1 and 2 in Chapter 9).

Undoubtedly, estrus was delayed in progestin-treated animals. The number of heifers showing behavioural estrus reached a peak before Day 20 after TAI in control heifers, and after Day 20 in progestin-treated heifers. In Experiment 1 (Chapter 9), the pregnancy rate in heifers resynchronized with a used-CIDR tended to be lower than in control heifers because estrus and conception rates were numerically lower. Delaying estrus in cattle destined to return to estrus before progestin withdrawal could compromise fertility through the development of persistent follicles. Persistent follicles develop as a result of the higher frequency of LH pulses that have been shown to occur during the period of treatment with used-CIDR inserts in animals in which luteolysis has occurred (Savio et al., 1993). The greater frequency of LH release or the greater secretion of

estradiol by the persistent follicle probably contributes to the reduced fertility. It has been reported that oocytes from persistent follicles were fertilizable but development of the resultant zygote usually stopped before the 16-cell stage (Ahmad et al., 1995). Increased concentrations of estradiol for an extended interval may also affect the oviductal or uterine environment so that embryonic development is compromised. Binelli et al. (1999) reported altered pattern of protein synthesis and secretion in the oviducts of cows with persistent dominant follicles, suggesting that an inappropriate oviductal environment may contribute to the lower fertility. However, when results from other studies are evaluated, the most likely cause for reduced fertility of cattle ovulating persistent follicles is abnormal oocyte development. Oocytes from persistent follicles were at more advanced stages of maturation than those from follicles of normal age and size (Revah and Butler, 1996; Mihm et al., 1999). Wehrman et al. (1996b) showed that the development of a persistent follicle prior to the synchronized estrus did not adversely affect conception rate in recipients that received frozen-thawed embryos 7 d after estrus, and this has been confirmed in our unpublished studies. In addition, Wehrman et al. (1996a) superstimulated normal cycling cattle and those with persistent follicles. Although persistent follicles suppressed superovulatory response, embryo quality did not differ between groups suggesting that the oviductal and uterine environments in cows with persistent follicles are not detrimental to embryonic development. Although the cause of abnormal oocyte competence in persistent follicle is currently unknown, as with estrus synchronization regimens, persistent follicles must be avoided in resynchronization schemes. Hence, treatments to reset the follicle wave were administered in the experiments involving resynchronization.

Although estrogens are highly efficacious in synchronizing ovarian follicle development, it is recognized that exogenous estrogens can induce luteolysis (Chapter 7). In this study (Chapter 9), the administration of 1.5 mg of E-17β and 50 mg of P at CIDR insertion did not adversely affect pregnancy rate following TAI, but the administration of 0.5 mg E-17β 24 h after CIDR removal was associated with a reduction in pregnancy rate following TAI. As indicated in the Discussion in Chapter 9, these data are difficult to rationalize. These results could be by chance, or luteolysis may have occurred in E-17β-treated heifers, or the administration of E-17β after CIDR removal may have resulted in the reinsemination of pregnant heifers. As only E-17β was used to synchronize LH release and ovulation, it is reasonable to conclude that the most likely cause of reduced fertility was reinsemination of pregnant heifers. However, only 1 to 4% of heifers detected in estrus and reinseminated following TAI were subsequently diagnosed pregnant in Experiment 1 and the field trial (Chapter 9). Embryonic or fetal mortality following AI of pregnant cattle has been estimated to range from 24 to 49% (Sturman et al., 1980; 2000). Based on these previous studies, the proportion of pregnant heifers detected in estrus and reinseminated would not have been more than 8% in our study. It is noteworthy that animals in Experiment 1 and the Field Trial received only CIDR inserts. Treatment with E-17β following progestin removal may have increased the incidence of behavioural estrus in pregnant animals.

Regardless of the reason for reduced fertility following TAI in estradiol-treated heifers, treatments designed to induce a new ovarian follicular wave in cattle with unknown pregnancy status must not disrupt ongoing pregnancies from a previous

insemination. Alternative treatments for the synchronization of follicle wave emergence in previously timed inseminated heifers were investigated. Although the administration of progesterone or GnRH did not adversely affect pregnancy rate following TAI, neither treatment increased estrus or conception rates in nonpregnant cattle. On the contrary, administration of 150 mg progesterone not only did not synchronize follicular wave emergence effectively, but also resulted in reduced expression of estrus and subsequent pregnancy rates to reinsemination. Administration of GnRH to synchronized follicular wave emergence was effective in more than 80% of cows without altering estrous cycle length. However, GnRH treatment did not increase conception rate in reinseminated heifers.

Overall, the proportion of nonpregnant cattle resynchronized with either a used-CIDR or MGA that showed behavioural estrus approached 80%, regardless of the estrus detection method utilized. In only one experiment was there more than 90% of non-pregnant heifers returning to estrus over an 8-d interval. Failure to detect estrus in heifers that were subsequently diagnosed to be nonpregnant could have been due to unobserved estrus or embryonic loss (and returning to estrus after the estrus detection had ceased). We attempted to increase the proportion of nonpregnant heifers showing behavioural estrus by giving estradiol after progestin withdrawal. However, there was no benefit of estradiol treatment after the termination of MGA feeding, or at the time of CIDR removal. The administration of estradiol tended to increase the percentage of nonpregnant animals showing behavioural estrus in some experiments, while it did not in others. When treatment with estradiol tended to increase the incidence of behavioural estrus

during the rebreeding period, there was a numerically decreased conception rate, resulting in similar pregnancy rates. Therefore, there was little benefit to the use of estradiol to increase the expression of behavioural estrus in nonpregnant heifers.

The use of short-term progestins to resynchronize cattle that did not conceive to the TAI is an important tool to increase the cumulative pregnancy rates while minimizing the amount of time needed for estrus detection. Because CIDR inserts could be re-used in estrus synchronization schemes without a detrimental effect on fertility (Chapter 8), we investigated the use of an orally active synthetic progestin (MGA), as an alternative to CIDRs for the resynchronization of TAI animals. MGA supplementation would be less expensive and require less handling of the animals than the reinsertion of used-CIDR. However, conception and pregnancy rates in nonpregnant heifers were consistently higher in heifers receiving a used-CIDR as compared to those fed MGA for resynchronization following TAI.

Synthetic and natural progestins, when administered at the dose used commercially for estrus synchronation, do not suppress LH pulse frequency to the extent that occurs in cattle during the mid-luteal phase of the estrous cycle (Kojima et al., 1995). Although estrus was delayed following the use of either progestin (CIDR and MGA), persistent follicles may have developed during the period of treatment. However, the reason for the further reduction in fertility that resulted from feeding MGA is unclear. Whether MGA treatment directly affects oocyte competence, oviductal or uterine

environment, and subsequent embryo development is unknown and should be addressed in future studies.

Although a detailed discussion of economics of protocols for TAI and resynchronization is not the purpose of this thesis, it is important to consider economic implications before implementing any resynchronization protocol. Based on Experiment 1 (Chapter 9), treatment with a previously-used CIDR resulted in a synchronous return to estrus with 61.7% of the heifers in estrus over 2 d. However, the insertion and withdrawal of CIDR takes another 2 d. Alternatively, if estrus detection and AI were limited to 4 d (18 to 21 d after TAI) in untreated-control heifers, estrus rate would have been 54.3%. However, fertility rates tended to be higher in untreated-control than in CIDR-treated heifers, and pregnancy rates would not have been different (40.0 and 38.2%, respectively). Therefore, from an economical point of view, estrus resynchronization protocols should be implemented only if estrus and conception rates are improved. In this regard, although not significant, the inclusion of estradiol treatment at CIDR insertion on Day 13 increased the proportion of heifers pregnant by 15% compared to untreated-control heifers (Experiment 2, Chapter 9).

12.0 GENERAL CONCLUSIONS

12.1 Synchronization studies for timed-AI

1. Administration of 1 mg estradiol cypionate resulted in follicular wave emergence, but synchrony was less precise than with 5 mg of estradiol-17β.
2. Treatment with 0.5 mg estradiol cypionate either at the time of CIDR removal or 24 h later effectively synchronized the LH surge and ovulation of the dominant follicle of a follicular wave synchronized with E-17β.
3. When estradiol-17β was used to synchronize follicular wave emergence, pregnancy rate did not differ in heifers treated either with estradiol cypionate at 0 or 24 h after CIDR removal or estradiol benzoate at 24 h after CIDR removal.
4. When GnRH or estradiol cypionate was given to synchronize follicular wave emergence, pregnancy rate in heifers treated with estradiol cypionate 24 h after CIDR removal was higher than in those treated with estradiol cypionate at CIDR removal or GnRH at TAI.
5. Although the flax seed diet had a significantly greater linolenic acid content than the control or sunflower seed diet, a beneficial effect on fertility was not clearly demonstrated.

6. Treatment with 5 mg EV resulted in luteolysis in norgestomet-implanted heifers, and a shorter interval from norgestomet implant removal to estrus and ovulation than treatment with 5 mg of estradiol -17β.
7. The interval from treatment to follicular wave emergence in heifers was longer and more variable following administration of 5 mg of EV than 5 mg of estradiol-17β. However, ovarian follicular wave emergence and synchrony of estrus and ovulation in CIDR-treated cows given 1 or 2 mg EV were similar to those given 5 mg of estradiol-17β.
8. The synchronization of follicle wave emergence with 5 mg EV for superstimulation in Holstein cows resulted in a lower number of transferable embryos than following the administration of 5 mg estradiol-17β.
9. Fertility following TAI was not different between cattle receiving a new or once-used CIDR, but pregnancy rate was reduced in cattle receiving a twice-used CIDR.
10. The addition of 100 mg progesterone to the estradiol treatment for the synchronization of follicular wave emergence did not affect pregnancy rates, regardless of whether a new or used CIDR insert was used.

12.2 Resynchronization studies for estrus detection and AI

1 Progestin treatment starting 13 ± 1 d after TAI resulted in synchronized returns to estrus; compared to untreated control heifers, the range in the interval to onset of behavioural estrus was reduced by 6 to 9 d.

2 Fertility was higher in nonpregnant heifers given a used-CIDR than those fed MGA for resynchronization following TAI.

3 Administration of 1.5 mg of E-17β and 50 mg of P at CIDR insertion on Day 13 ± 1 d after TAI plus 0.5 mg E-17β 24 h after CIDR removal was associated with a reduction in pregnancy rate following TAI.

4 Administration of progesterone, ECP or GnRH to synchronize follicle wave emergence in nonpregnant heifers did not adversely affect pregnancy rate following TAI; however, only GnRH synchronized follicular wave emergence in cows. Although the administration of GnRH 13 ± 1 d after TAI did not alter estrous cycle length, it did not result in increased conception rates in reinseminated heifers.

5 Administration of estradiol at progestin removal tended to increase the percentage of nonpregnant cattle showing behavioural estrus in some experiments, but it resulted in a numerically decreased conception rate, and a similar pregnancy rate as untreated control cattle.

13.0 SUGGESTIONS FOR FUTURE RESEARCH

The studies reported in this thesis offer the background for the formulation of new hypotheses and the design of future studies for alternatives approaches for estrus synchronization and improvement of fertility following TAI.

In a short term, there would be a necessity to develop alternatives for the synchronization of follicular wave growth. There are no estradiol preparations on the veterinary market in North America at this time, and the European Union has indicated that estrogen and progesterone preparations will be removed from the veterinary market by 2007. Other estrus synchronization protocols ("Synch" family), which do not include the use of steroid hormones, are currently applied in cattle with variable results, particularly in heifers. For these reasons, the use of alternative approaches to the synchronization of follicular wave emergence, follicle growth and ovulation should be addressed in future studies on estrus synchronization for TAI.

Inhibin-A and other follicular secretions would be interesting candidates that need to be evaluated for the synchronization of follicular wave emergence. Steroid-free bovine follicular fluid treatment suppressed the peri-ovulatory FSH rise and follicular wave emergence in inhibin-immunised heifers (Wood et al., 1993). In addition, it has been shown that steroid- or inhibin-depleted follicular fluid can inhibit follicular development

in sheep (Campbell et al., 1991; O'Shea et al., 1994) and cattle (Law et al., 1992). These findings suggest that other factors (other than steroids and inhibin) present in follicular fluid, may affect gonadotrophin secretory patterns and in turn, follicular dynamics. Indeed, several other non-steroidal factors such as the IGF family (Spicer and Echternkamp, 1995), Epidermal growth factor (EGF; Rall et al., 1985), Fibroblast growth factor (FGF; Gospodarowicz et al., 1987), Gonadotrophin surge-attenuating factor (GnSAF; Fowler et al., 2003,), Follistatin (Shimasaki et al., 1988), the Transforming growth factor-β superfamily (TGF-β; Knight and Glister, 2003) which includes Activin (Vale et al., 1986), Bone morphogenetic proteins (BMP; Shimasaki et al., 1999), and Growth differentiation factors (GDF; Eppig, 2001), and emerging new factors such as Kisspeptins (Gottsch et al., 2004) have been shown to exist in follicular fluid, hypothalamo and pituitary cells . An improved understanding of how these various local factors, steroids and gonadotrophin secretions are integrated into a coordinated response in terms of follicular development to ovulatory status will improve our ability to control follicular growth.

Ovarian steroid hormones, primarily progesterone, control LH release from the anterior pituitary. In cattle, LH secretion is essential to maintain ovarian follicular function (i.e. steroidogenesis) during the estrous cycle. Cortisol is a steroid hormone with a similar molecular structure as progesterone. Acute increases in defined stress levels of cortisol have been shown to rapidly and robustly suppress pulsatile LH secretion in ovariectomized ewes (Debus et al., 2002). However, cortisol did not suppress GnRH secretion (Breen and Karsch, 2004), indicating that the site of disruption of LH secretion

is at pituitary level. Cortisol might also act at the level of the ovary; glucocorticoid receptors have been identified in granulosa cells of the rat (Schreiber et al., 1982). Despite all the evidence that elevations in glucocorticoids will inhibit reproductive neuroendocrine activity and folliculogenesis in animals through suppression of LH release, the use of cortisol to manipulate ovarian follicular development has not been studied. Studies are warranted to determine whether elevations in cortisol will interfere with the expression of endocrine events and follicular dynamics in cattle.

Even though the use of androgens to synchronize follicular wave development in food animals may be of some concern, testosterone is another candidate that could be investigated. In rats, high concentrations of androgens interfered with FSH and estrogen mediated events, which are critical for follicle development, and thereby promoted follicle atresia (Farookhi, 1980). Testosterone provides the primary negative feedback signal controlling LH secretion in bulls (Schanbacher et al., 1983). In Holstein heifers, persistent follicles maintained with norgestomet implants regressed following four consecutive treatments with 200 mg of testosterone (Rajamahendran and Manikkam, 1994). Following testosterone injections, heifers had LH profiles that were not different from those in control heifers, indicating that testosterone may have caused atresia by decreasing basal FSH concentrations or through a direct effect on the persistent dominant follicle (Rajamahendran and Manikkam, 1994). The effect of acute treatment with testosterone on endocrine parameters and follicular dynamics in cattle has not been studied.

Previous studies with ovariectomized cattle have shown that following the insertion of a new CIDR, plasma progesterone concentrations were elevated to more than 6 ng/mL for 48 h before plasma progesterone concentrations stabilized at less than 3.5 ng/mL (Macmillan and Peterson, 2003; Martínez et al., 2003a) However, when estradiol was given at the time of CIDR insertion, estradiol-induced LH release was not blocked, even though 100 mg progesterone was injected concurrently with estradiol (Martínez et al., 2003a). Furthermore, low plasma progesterone concentrations during an estrus synchronization treatment protocol has been associated with reduced fertility, indicating that stage of the estrous cycle at initiation of estradiol-progesterone treatment may be an important factor. Presynchronization with PGF or progestins before implementing estradiol/progesterone-based estrus synchonization schemes for TAI may improve pregnancy rates. Further studies are needed to characterize the effect of estradiol and progesterone treatments on follicular dynamics and fertility in cattle treated at various stages of the estrous cycle.

Pulsatile secretion of GnRH from the hypothalamus is regulated by circulating progesterone concentrations during the estrous cycle (Kinder et al., 1996). The frequency of GnRH pulses, which is determined by progesterone concentrations, will determine the frequency of pulsatile secretion of LH from the anterior pituitary. It is known that long-term progestin-treated cattle can develop a large dominant follicle with a prolonged lifespan, which secretes more estradiol and inhibin. However, it is not clear whether the reduction in fertility in cattle with persistent follicle is due to effects of estrogens, LH, or both. Although there is evidence that over-exposure of follicles to LH may be detrimental

to oocyte/embryo quality in cattle (Murphy et al., 1984), it is unknown whether acute changes in the frequency of LH pulses will affect the developmental competence of oocytes in growing follicles. Future studies should be aimed at determining the developmental competence of oocytes from follicles that develop under different circulating concentrations of progesterone, estradiol and gonadotrophins.

Mechanisms of physiological and pharmacological control of ovulation and their relationship with endocrinology, follicular development and the environment need to be systematically investigated. The effect of ovulatory follicle size on fertility in estrus synchronization programs is been currently investigated. However, studies should be designed to evaluate the effect of hormonal treatments and ovulatory follicle size on the temporal relationships of estrus, the LH surge, ovulation, oocyte competence, fertilization, embryo development, and CL function. Oocytes from preovulatory follicles induced by different hormonal treatments may have different developmental capacity due to changes in the follicular fluid as a result of changes in levels of circulating hormones. Oocyte competence may be also related to the interval from follicular wave emergence to luteolysis, or the interval from luteolysis to ovulation, and should be further investigated.

Studies should also be directed to the effect of current hormonal treatment protocols to synchronize ovulation on early luteal development. One important characteristic of early luteal development is the rapid rate of cell proliferation, primarily of endothelial cells, associated with the growth of follicular-derived tissue into a CL. Several in vitro studies have shown that early CLs are capable of producing progesterone

levels similar to midcycle CLs, suggesting that the early CL is functionally active. This is consistent with the fact that luteinization and CL development are accompanied by extensive angiogenensis (Smith et al., 1994), which correlates with an increased rate of luteal blood flow and progesterone production. The CL has an extensive capillary network and endothelial cells contribute to approximately 45% of the total luteal cell composition (Farin et al., 1986). Inhibited development and function of the CL has been associated with suppression of luteal angiogenesis. Hence, it is likely that any inadequacy in luteal angiogenesis would reduce progesterone production, which in turn could lead to reduced fertility. Vascular endothelial growth factor (VEGF) is a potent angiogenic factor that has been identified in the CL of many species including cattle (Berisha et al., 2000). Human and equine chorionic gonadotrophins (two hormone preparations used in estrus synchronization programs in ruminants) have been shown to stimulate the ovarian production of VEGF in vitro (Christenson and Stouffer, 1997; Laitinen et al., 1997). Whether hormone preparations, used to induce an LH surge and ovulation in estrus synchronization programs, would affect luteal angiogenesis has not been investigated.

Cumulus expansion is one of several processes that must occur in preovulatory follicles to enable ovulation and perhaps fertilization (Eppig, 2001). Follicle-stimulating hormone has been shown to stimulate the expansion of cumulus cells isolated from mice, rats and pigs in vitro (Dekel and Phillips, 1979; Eppig, 1979; Hillensjo and Channing, 1980). Preovulatory estradiol concentration seems to be related to the preovulatory plasma gonadotrophin surge; it has been suggested that GnRH-induced preovulatory plasma FSH and LH concentrations are decreased in cows with low plasma estradiol

concentrations but not in cows with high plasma estradiol concentrations (Gilad et al., 1993). Hence, the secretory pattern of FSH and LH may be important to achieve high fertility. The developmental capacity of oocytes collected from the preovulatory follicle before and after an endogenous or induced gonadotrophin surge should be addressed in future studies.

Resynchronization protocols presented here synchronized estrus in cattle nonpregnant following TAI and facilitated estrus detection and reinsemination. However, fertility and particularly the number of open cattle observed in estrus, need to be increased. Treatments utilized here did not consistently increase these two reproductive parameters; therefore, the use of alternative protocols should be investigated in future studies.

Late embryonic mortality, in particular that occurring between maternal recognition of pregnancy and pregnancy diagnosis, reduces the proportion of cattle returning to estrus following TAI. However, a means of increasing estrus rate in nonpregnant cattle may be to induce luteolysis as early as possible following pregnancy diagnosis. Methods of early pregnancy diagnosis (before day 21 after TAI) would allow for the administration of exogenous PGF in those animals diagnosed nonpregnant before or around the time of endogenous PGF release. Early diagnosis of nonpregnant animals would also reduce the number of animals that need to be observed, permitting a more assertive and intensive estrus detection. A current alternative is to perform pregnancy diagnosis by ultrasonography on Day 26-28 following TAI and treat all nonpregnant

animals with PGF. Administration of GnRH to synchronize follicular wave emergence can be given 7 days earlier, providing the possibility of a second TAI. However, a high proportion of lactating cows treated with GnRH on Day 21 displayed sign of estrus prior to pregnancy diagnosis and TAI (Chebel et al., 2003). Whether the addition of a CIDR would affect the proportion of cows that complete the TAI protocol should be investigated.

The knowledge acquired from experiments addressing factors that affect the developmental capability of oocytes, oviductal and uterine environment, embryo development and CL function after hormonal treatments will provide the means by which reproductive function can be controlled or manipulated, and make it possible to explain fertility observed in the field. Identification of these factors will provide the means for the development of strategies by which pregnancy rates can be optimized in estrus synchronization schemes for TAI.

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