SYNCHRONIZATION OF FOLLICULAR WAVE DYNAMICS AND OVULATION FOR FIXED-TIME ARTIFICIAL INSEMINATION IN CATTLE

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ABSTRACT

The overall objective was to develop new synchronization protocols that facilitate fixed-time artificial insemination (AI). A series of experiments were designed to evaluate the effects of estradiol and progesterone on gonadotrophin release, follicular wave emergence and ovulation in beef cattle. In ovariectomized cows, a new CIDR-B device increased plasma progesterone to near-luteal concentrations, but for only 2 to 3 days. Injection of 100 mg progesterone increased plasma progesterone approximately 2 ng/mL. Progesterone suppressed plasma LH concentrations but did not affect plasma FSH concentrations. Estradiol, with or without progesterone, resulted in FSH suppression with resurgence (and follicular wave emergence) at an interval that varied according to the estradiol formulation. Estradiol administration following CIDR-B removal resulted in LH release (and ovulation in intact animals). Both estradiol-17β and estradiol benzoate (EB) synchronized ovarian follicular wave emergence in CIDR-B-treated animals and the interval from CIDR-B removal to ovulation (72 to 120 h) was shorter and more synchronous in estradiol-treated animals than in controls. In cattle given a CIDR-B device and estradiol plus progesterone, estradiol treatment following CIDR-B removal 7 days later resulted in acceptable conception rates to fixed-time AI. Estradiol or GnRH at the beginning and end of a 7-day MGA-based synchronization regimen resulted in acceptable pregnancy rates to fixed-time AI. In a single experiment, EB, GnRH or pLH in CIDR-B- or MGA-treated beef heifers effectively synchronized ovulation for fixed-time AI. Pregnancy rates were, on average, 58.0% (range 52.5 to 65.0%). A 6- or 7-d interval from GnRH to PGF in a Cosynch regimen resulted in similar pregnancy rates in cows. The addition of a progestin to a Cosynch or Ovsynch

regimen improved pregnancy rates in heifers but not in cows. Synchronization of follicular wave emergence and ovulation in a two-dose PGF-based protocol resulted in acceptable fertility to fixed-time AI; the administration of EB induced luteal regression in some animals but E-17β did not. In conclusion, synchronization programs including GnRH, pLH or estradiol to synchronize follicular wave emergence and ovulation in CIDR-B-, MGA- or two-dose PGF-protocols resulted in acceptable pregnancy rates to fixed-time AI.

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DEDICATION

I dedicate this thesis to my parents, Florencio and Enriqueta, who are my examples of loyalty and humility, my sisters Nora and Maria Florencia, and to my dear deceased brother, Angel. I also dedicate this thesis to Nico, Valentina, Rosa, and Antonio and all my family.

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LIST OF ABBREVIATIONS

µg	microgram
AI	artificial insemination
CIDR-B	controlled internal drug release for use in the bovine species
CL	corpus luteum
cm	centimeters
E-17β	estradiol-17β
EP	estradiol-17β plus progesterone
EB	estradiol benzoate
EV	estradiol valerate
FA	follicular ablation
FSH	follicle stimulating hormone
GnRH	gonadotrophin releasing hormone
h	hours
i.m.	intramuscularly
IOI	interovulatory interval
kg	kilogram
LH	luteinizing hormone
mg	milligram
MGA	melengestrol acetate
mL	milliliter
mm	millimeter
P	progesterone

PGF	prostaglandin $F_{2\alpha}$
pLH	porcine luteinizing hormone
PRID	progesterone releasing intravaginal device
S	South
SD	standard deviation
SEM	standard error of the mean
SMB	Syncro-Mate-B
W	West
WE	wave emergence

1.0 GENERAL INTRODUCTION.

The greatest genetic progress in cattle production has been made through the use of artificial insemination (AI), especially in dairy herds. The use of AI for genetic improvement involves the use of semen from genetically superior and highly selected bulls, while genetically superior bulls and cows are used in the embryo transfer industry. The utilization of AI has increased the number of cows of high genetically merit. For instance, the genetic improvement of Holstein cattle for milk production through AI has increased about 5 times since 1980 (Foote, 1996).

Estrus synchronization in cattle has been linked to AI programs. It is also recognized that the widespread use of AI depends on effective methods to control the estrous cycle. Although many methods of synchronization have been developed, only 5% of beef herds in Canada use AI, while in dairy cattle the proportion is 72% (Vetrepharm Can Inc). The development of new methods of manipulation of the bovine estrous cycle for fixed-time AI is intended to increase those proportions, particularly in beef cattle.

Knowledge of the bovine estrous cycle has increased considerably due to the improvement of techniques such as hormone measurements, real-time ultrasonography to observe the changes in reproductive organs as they occurred (Pierson and Ginther,

1984, 1988) and magnetic resonance imaging used to detect subtle changes in the molecular composition of the ovaries (Hilton et al., 2000).

The scientific literature concerning estrus synchronization, AI and embryo transfer has multiplied as basic knowledge about the estrous cycle in cattle has increased. Control of the estrous cycle has gone from the control of the luteal phase to include the control of follicular growth with ovulation occurring at predictable times, which may facilitate the use of fixed-time AI. The knowledge of ovarian dynamics and improvement of synchronization protocols will raise producers' confidence and lead to an increased use of AI.

2.0 REVIEW OF LITERATURE.

2.1 Physiology of the estrous cycle.

2.1.1 Follicular growth in cattle.

Follicular growth begins in the fetus before parturition. By mid-gestation, the fetal ovary bears *primordial* follicles which contain oogonia. A bovine female is born with a complete collection of oogonia in primordial follicles that have only a single flat cell layer (Erickson, 1966, Wiltbank, 1997). There are on average 133,000 primordial follicles in the ovary of a bovine female (Erickson, 1966). Primordial follicles are the source of future follicles during a female's life. The recruitment process begins with the

formation of *primary* follicles in which the surrounding cells become cuboidal and proliferate (granulosa cells). When granulosa cells multiply and many cell layers surround the oocytes, the follicle is called a *secondary* follicle. Intercellular cavities develop which are filled with follicular fluid. The cavities converge and conclude with the formation of a follicular antrum; the follicle is called an antral or *Tertiary* follicle. Fully-grown follicles are called Graafian and become preovulatory after the first preovulatory gonadotrophin surge and before the first ovulation (onset of puberty). In the reproductive life of the female, a small number of follicles ovulate, but most become atretic.

Growth of follicles under 2 mm is thought to be independent of gonadotrophins. *In vitro* growth of primordial follicles has been observed in the absence of gonadotrophins in serum-free media (Braw-Tal and Yossefi, 1997). However, there is evidence that FSH receptors are functionally active during preantral development; granulosa cells increased in number and there was more thymidine uptake after being stimulated with FSH in serum-free cultures of bovine oocytes (McNatty et al., 1999). The process of growth and further differentiation of a primordial follicle into an ovulatory follicle can take as long as 60 days (Lussier et al., 1987). Antral follicles equal to or greater than 2 mm in diameter are responsive to gonadotrophins (Webb et al., 1999), as demonstrated by the exhibition of waves of follicular growth. Follicular waves begin at a very early age, in 2-week old calves through puberty (Adams et al., 1994a; Evans et al., 1994b).

The hypothesis that the growth of ovarian follicles in cattle occurs in a wave-like fashion, and that two waves of follicular activity take place during the bovine estrous

cycle was postulated by Rajakosky (1960), based on studies of slaughterhouse ovaries. This hypothesis was confirmed by daily serial observation of the ovaries using real-time ultrasonography (Pierson and Ginther, 1984). A wave of follicular growth involves the synchronous development of a group of follicles (8 to 40; Adams, 1999) and is characterized by a dominant follicle and several subordinates. It has been well established that a bovine cycle regularly consists of two or three follicular waves (Ginther et al., 1989a, 1989b). Follicular waves also occur in pregnant (Ginther et al., 1996a), postpartum (Rajamahendran and Taylor, 1990; Savio et al., 1990a, 1990b) and even prepubertal (Evans et al., 1994a, 1994b; Adams et al., 1994a) cattle.

Many stages of follicular growth have been clearly characterized. Some definitions are generally accepted to define follicular developmental events. For instance, *recruitment* involves gonadotrophin stimulation of a pool of growing follicles. This is followed by *selection*, whereby one of these recruited follicles is favored to continue its further growth becoming a dominant follicle and exerting suppression of its subordinates. This process is called *dominance*. With ultrasonography, the emergence of a follicular wave is typically detected when follicles 4 or 5 mm in diameter appear in increased numbers in the ovaries compared with what was observed in a previous scan. It is possible to retrospectively identify the dominant follicle at the time of wave emergence (by examining diameter and location of the largest follicle during consecutive days). When the corpus luteum (CL) regresses, the dominant follicle of that follicular wave becomes the ovulatory follicle. Ovulation can be also detected by ultrasonography as the disappearance of the dominant follicle observed in the first of two consecutive observations.

An interovulatory interval (IOI) is the period between two sequential ovulations. In this regard, different laboratories have found a predominance of 2-wave IOI (Ginther et al., 1989a, 1989b), whereas others observed mostly 3-wave IOI (Savio et al., 1988; Sirois and Fortune, 1988). Two-wave IOI (20.4 ± 0.3 days) were shorter ($P<0.01$) than those with three follicular waves (22.8 ± 0.6 days; Ginther et al., 1989b). Nevertheless, length of the IOI or number of waves did not affect pregnancy rates (Cooperative Regional Research Project, 1996). Follicular waves usually emerge on Days 0 and 10 in 2-wave cattle, and on Days 0, 9 and 16 in 3-wave cattle (Ginther et al., 1989a). Every follicle has 3 phases of growth. The dominant follicle grows linearly for 5 days (growing phase), determined retrospectively from the first detection of the dominant follicle to the day of cessation of progressive increase in diameter. It remains at approximately the same size for 5 to 7 days (static phase), and then begins to regress by decreasing size until it is no longer identifiable (regressing phase; Ginther et al., 1989a). The dominant follicle exerts suppression on subordinate follicles, which cease to grow within a few days after the emergence of a follicular wave (Ko et al., 1991; Adams et al., 1993a, 1993b), and a new follicular wave does not appear while the dominant follicle is in its growing phase or initial period of its static phase (Adams et al., 1993a). Elimination of the dominant follicle results in a new follicular wave (Adams et al., 1993b). Selection is a dynamic process occurring during the growing phase of the dominant follicle associated with follicular divergence (beginning of the greatest difference in growth rate between the two largest follicles; Ginther et al., 1996b, 2000). It has been suggested that follicular divergence (or deviation) is an important event in

the selection of a dominant follicle (Ginther et al., 2000). It occurs when plasma FSH concentrations decrease and the dominant follicle grows linearly.

As mentioned previously, follicular waves also occur in prepubertal animals and their characteristics have been recently studied (Evans et al., 1994a, 1994b; Adams et al., 1994a). The diameter profiles of the dominant and largest subordinate follicles were found to increase with age; being the greatest between 2 to 8 and 24 to 40 weeks. The IOI after the first ovulation was short (7.7 ± 0.2 days), containing only one follicular wave and the CL resulting from the first ovulation was smaller and shorter-lived than the CL of subsequent IOI (Evans et al., 1994a; Adams, 1998). The second IOI (20.3±0.5 days) was similar in length to the subsequent IOI (Evans et al., 1994a). It has been reported that all mechanisms controlling follicular wave dynamics in sexually mature heifers were present in 36-week-old prepubertal heifers (Adams, 1999).

Follicular dynamics in postpartum animals resembles those in peripubertal heifers. The first follicle wave emerged 10 to 14 days after calving (Murphy et al., 1991). In dairy cows, the post-partum interval to detection of the first dominant follicle was shorter in autumn (6.8±1.8 days) than in spring (20.0±10.1 days; Savio et al.; 1990a). In contrast, the respective intervals after parturition to ovulation were very similar (approximately 27 days). When the first ovulatory follicle was detected within 9 days from parturition, the subsequent IOI was normal in length (18 to 24 days) or long (>24 days) compared to the length of subsequent estrous cycles; however, when the ovulatory follicle was identified after 20 days from parturition, the subsequent IOI was short (9 to 15 days, Savio et al., 1990b). In beef cattle, the first IOI was almost always short and all subsequent cycles were of normal length (19-23 days; Adams, 1998).

2.1.2 Endocrinology of the estrous cycle.

The hormonal component of the estrous cycle is ruled by secretions from the hypothalamo-hypophyso-gonadal axis. Gonadotrophin releasing hormone (GnRH) dictates the synthesis and secretion of both luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary gland (Herbison, 1997). GnRH is produced by specialized neurons in the hypothalamus and released in pulses into a portal blood system that connects the hypothalamus with the pituitary. The pulsatile characteristic of GnRH secretion is necessary to prevent down-regulation of GnRH receptors (Roche et al., 1997) and determines the pulsatile pattern of LH release. However, FSH secretion is passive and only partially regulated by GnRH. There are other factors and hormones that coordinate FSH release, i.e., activin, inhibin, follistatin, estradiol. Increases in inhibin are associated with emergence of each follicular wave, whereas estradiol increases significantly during the first and last follicular waves. Inhibin seems to reduce FSH to a level that maintains species-specific numbers of ovulations (Taya et al., 1996)

The length of the estrous cycle and the hypothalamic secretion of GnRH are regulated by progesterone from the CL. There is a strong negative feed-back on GnRH during mid-cycle, when plasma progesterone concentrations are high, resulting in a decreased mean concentration and pulse frequency of LH. Late in the estrous cycle, when prostaglandin F2α (PGF) is secreted from the uterus, progesterone concentrations are reduced in the systemic circulation, and both mean concentration and pulsatility of LH increase. At the same time, the future ovulatory follicle is acquiring LH receptors on granulosa cells, which results in increased estradiol concentrations, and in turn, estrous

behaviour. The elevated estradiol results in a further increase in GnRH pulses and the generation of a GnRH surge followed by a peak in LH concentrations (Bryner et al., 1990), inducing ovulation of the preovulatory follicle. The increase in GnRH pulses and the LH peak are concurrent with the elevation of FSH that will induce the first follicular wave. The FSH surge concurrent with the LH peak that will induce ovulation has been considered the first FSH surge (Bergfelt et al., 1997). A second FSH surge occurred around the time of ovulation and this coincided with the emergence of the first follicular wave (Wiltbank et al., 2002). Peak concentrations of FSH and LH occurred simultaneously in 92% of heifers; FSH levels reached their maximum before and continued high until after the LH peak (Bergfelt et al., 1997). Each follicular wave was preceded by an FSH surge and there were 2 or 3 FSH surges during the estrous cycle, in accordance with the number of follicular waves that a female has between two consecutive ovulations (Adams et al., 1992a). Therefore, progesterone regulates LH release whereas estradiol and inhibins regulate FSH secretion in cattle. A specific hormonal pattern will determine the number of follicular waves during the cycle and the time of ovulation.

2.1.3 Relationship between hormonal and ovarian events.

Interactions between local and systemic hormones and other ovarian factors during the estrous cycle results in ovulation of the oocyte contained in the dominant follicle of the last follicular wave. Adams et al. (1992a) reported that FSH was temporally related to the growth of small follicles. There are recurrent and transient increases in FSH concentrations (surges) that will induce the emergence of a follicular wave approximately one day later (Adams et al., 1992a). Mean FSH concentrations increased 8 h before wave emergence and remained elevated for 8 h after wave emergence (Ginther et al., 1998). This was followed by a decrease in FSH, which became significant within an interval of 24 h starting 40 h before follicular growth divergence, which occurred on average 2.8 days after emergence of the follicular wave (Ginther et al., 1998). The initial decline in FSH began when the largest follicles were 6 mm in diameter (Ginther et al., 1996b). As FSH declined, follicles of the wave continued to grow, resulting in increasing estradiol secretion. These growing follicles ≥4 mm in diameter contain aromatase, an important enzyme of the estradiol production pathway (McNatty et al., 1999).

All follicles greater than 5 mm in diameter contribute to the FSH decline and any 5 mm follicle is capable of becoming dominant (Gibbons et al., 1997; 1999). As follicles continue to grow, FSH is still required by follicles, but its concentration continues to decline (Ginther et al., 2001a). Divergence in follicular growth occurs 2.8 days after follicular wave emergence and is manifested by a continued growth of the dominant follicle and reduction or cessation of growth of subordinate follicles. Growth divergence begins when the largest follicle is 8.5 mm in diameter (Ginther et al., 1996b; Kulick et

al., 1999). Treatment with estradiol resulted in a transient decrease of FSH and an accompanying decline of follicles greater than or equal to 8.5 mm in diameter, which indicates FSH dependence of the dominant follicle around the time of growth divergence (Ginther et al., 2000). However, once the dominant follicle has completed the process of selection, and granulosa cells have acquired LH receptors (Xu et al., 1995), follicular growth divergence has occurred. The dominant follicle acquires LH receptors and survives in an environment of declining concentrations of FSH. When the dominant follicle is selected, LH stimulates the production of estradiol and insulin-like growth factor by follicular cells (Ginther et al., 2001a, b), increasing capacity to suppress FSH (Ginther et al., 2000). Concentrations of FSH are lower than those required by subordinate follicles (Ginther et al., 2000) and thus regress. This suppression of FSH is maintained until the dominant follicle loses ability to produce estradiol (late static phase) or ovulates.

Elevated concentrations of LH and reduced concentrations of FSH were already present at the beginning of follicular growth divergence (Ginther et al., 1998). The dominant follicle apparently exerts suppression through the production of hormones (estradiol, inhibin, activin, follistatin), and other secretory products which may act locally or systemically to suppress the growth of subordinate follicles (Lucy et al., 1992). As an evidence of suppressive follicular factors, the injection of steroid-free bovine follicular fluid suppressed follicular growth while the treatment was administered (Kastelic et al., 1990b).

The dominant follicle has also been reported to generate a direct intra- or interovarian effect that inhibits follicle recruitment (Wolfsdorf et al., 1997). However,

all growing and viable follicles are capable of becoming dominant; a subordinate follicle has been shown to achieve dominant follicle status after ablation of the former dominant follicle (Adams et al., 1993a). In addition, follicles recruited at the beginning of the first and second follicular waves are capable of responding to superstimulatory treatments (Adams et al., 1994b). It has also been clearly shown that ovarian responses are greater when superstimulation is prior to selection of the dominant follicle than when superstimulation is initiated after dominance is established (Nasser et al., 1993). Therefore, those studies explained that, prior to selection, any follicle in a wave is capable of obtaining ovulatory status and after selection, larger subordinates are capable of reaching ovulatory status. However, due to declining levels of FSH, only one is selected.

2.2 Control of the length of the estrous cycle.

The bovine estrous cycle can be shortened or lengthened by the use of hormones such as estradiol and prostaglandin or progestins that mimic physiological events in the cycle. Historically, the control of the estrous cycle in cattle has involved two very different approaches. The first included protocols characterized by the control of luteal phase (without a accurate knowledge of follicular dynamics). The second (and more recent) approach involves the manipulation of both luteal and follicular dynamics. Advances had been slow since the first attempts to control the bovine estrous cycle by progesterone (Christian and Casida, 1948) or estrogens (Wiltbank et al., 1961) or the combination of both (Wiltbank et al., 1965). New approaches to the manipulation of the

bovine estrous cycle have been applied without impairment of fertility (Adams, 1998; Roche et al., 1997, Thatcher et al., 2001).

2.2.1 Prostaglandins.

Secretion of prostaglandin $F_{2\alpha}$ (PGF) by the nonpregnant uterus is responsible for terminating the luteal phase in the cow by causing regression of the CL. However, PGF is released by the endometrium as a result of oxytocin pulses from the posterior lobe of the pituitary. Estradiol produced by the dominant follicle of the last follicular wave also stimulates the action of oxytocin by inducing synthesis of oxytocin receptors. The process of uterine-induced luteal regression involves a veno-arterial pathway (the uterine vein and the adjacent ovarian artery) by which PGF reaches the CL to induce luteolysis in sheep (Del Campo and Ginther, 1973; Mapletoft and Ginther, 1974), cattle (Ginther, 1974) or pigs (Del Campo and Ginther, 1973).

After approximately 14 days of progesterone secretion, the endometrium secretes 6-h pulses of PGF for approximately 36 h and PGF concentrations increased in uterine secretions and uterine vein plasma (Hansel et al., 1975). In one of the first studies involving PGF, a daily intrauterine infusion of 0.5 mg PGF ipsilateral to the CL between Days 5 and 16 of the estrous cycle resulted in most of the females treated showing estrus in the morning of the third day after treatment but on Days 1 to 4 after estrus was not effective (Rowson et al., 1972). Similar results were observed in another study in which heifers treated on Days 6 to 9 or 13 to 16 responded by regression of the CL confirmed by rectal palpation, while heifers treated 2, 3 or 4 days after estrus had a luteal life span

of 15 to 19 days (Lauderdale, 1972). These early studies suggested that the response to PGF treatment depends on the stage of the estrous cycle at which it is applied and that the CL was not responsive to a single injection of PGF during the first 5 days post-estrus. Responsiveness to PGF has been shown to increase progressively from 6 to 8 days post-estrus and the CL remains highly responsive for the remainder of diestrus (Momont and Seguin, 1984). After PGF treatment of beef heifers on Days 7 to 20 after estrus, the length of the estrous cycle was significantly reduced and pregnancy rates following estrus detection and AI (72.5%) did not differ from that in controls (73.0%; Roche, 1974).

The interval from PGF treatment to estrus has also been shown to depend on the stage of development of the dominant follicle present at the time of treatment. A study that involved the administration of PGF on different days of the estrous cycle (Days 3 to 10 and 7 to 16) resulted in different intervals from treatment to estrus. It was attributed that varying intervals depended on growth or atresia of large antral follicles, but no conclusions were stated (Macmillan and Henderson, 1984). In a more recent study, the effects of day of PGF treatment were examined based on the selection and development of the ovulatory follicle (Kastelic et al., 1990a). The interval from PGF treatment to ovulation was shorter in heifers treated on Day 5 after ovulation (growing/early static; 3 days) than in those treated on Day 12 (regressing phase of Wave 1 or early growing phase Wave 2, 4.5 days). Treatment on Days 5 or 8 was followed by ovulation of the dominant follicle of Wave 1 (near its maximum diameter at the time of treatment), whereas treatment on Day 12 was followed by ovulation of the dominant follicle of Wave 2; since this follicle was small at the time of treatment, required more time to

reach ovulatory size. It was concluded that the viable dominant follicle present at the time of PGF treatment grew to its ovulatory size in all stages of follicle development. The interval from PGF treatment to estrus will depend on the time of growth up to the ovulatory size. This study established the rationale for synchronization of follicular waves prior to inducing luteal regression in estrus synchronization programs.

2.2.2 Progesterone and progestins

Chemically classified as a steroid, progesterone is one of the most important hormones that have been used to control the estrous cycle in cattle. It was first used in 1948 to alter the length of the cycle (Christian and Casida, 1948). Yearling heifers were treated with daily injections of 25 or 50 mg of progesterone for 14 days, starting on the Day 14 of the estrous cycle. Although estrus and ovulation were prevented in heifers treated with 50 mg of progesterone, heifers came into estrus 5 or 6 days later after suspension of treatment. The dose of 25 mg of progesterone was effective in suppressing estrus in all treated heifers, but ovulation was prevented only in 50% of heifers (Christian and Casida, 1948).

Later studies demonstrated that progesterone or progestins given for a term longer than the normal life span of the CL (i.e., >14 days) resulted in synchronous estrus after treatment was discontinued, but fertility was poor (Trimberger and Hansel, 1955; Wiltbank et al., 1965). Recently, it has been shown that "persistent follicles" arise because of an increased LH pulsatility, which occurs when the CL regresses, but ovulation was prevented by the low level of progesterone administered (Cooperative

Regional Research Project, 1996). The administration of progesterone suppresses LH pulse frequency if given to cattle bearing a CL, which in turn, causes suppression of dominant follicle growth in a dose-dependent fashion (Adams et al., 1992b; Sirois and Fortune, 1990). However, the dose of progestins used to control the estrous cycle in cattle (melengestrol acetate, MGA, in the feed or progesterone-impregnated devices, PRID or CIDR-B) has relatively less suppressive effects on LH secretion than the CL of the cycle and following luteal regression, the increased LH pulse frequency results in 'persistent' follicles (Custer et al., 1994; Kojima et al., 1995; Savio et al., 1993). Persistent follicles contain aged, infertile oocytes (Custer et al., 1994; Mihm et al., 1994, Revah and Butler, 1996) and insemination following progestin withdrawal results in poor fertility (reviewed in Larson and Ball, 1992 and Odde, 1990).

The progesterone-impregnated CIDR-B (controlled internal drug release) intravaginal device has been recently approved in Canada for synchronization of estrus in beef cattle. Label directions (for AI) state that the device should be placed in the vagina for 7 days; PGF is to be given 24 hours before device removal and estrus is detected for 3 days, starting 48 hours after device removal. The insertion of a CIDR-B device increased progesterone concentrations 2 h after insertion, remaining high for at least 48, and decreased very slowly until the day of CIDR-B removal (Macmillan et al., 1991). However, the CIDR-B device has been also used with different approaches used to synchronize follicular development. MGA is the other commercially available orally administered progestin in the Canadian market. The most common MGA-based estrus synchronization protocol consisted of 14 days of MGA feeding followed by an injection of PGF 17-19 days later, with insemination at detected estrus (Lamb et al., 2000). There

is a recent trend for the utilization of MGA in short-term protocols for estrus synchronization. However, the risk of persistent follicles and low fertility still remains when there is no attempt to control follicular growth. Short-term MGA programs that included treatment with estradiol or GnRH to synchronize follicular wave emergence have been designed (Kastelic et al., 1996, 1997; Thundathil et al., 1999). Estradiol-17β treatment at the beginning of an MGA protocol in beef cattle resulted in more consistent estrus and pregnancy rates (to AI after detected estrus) than GnRH or Controls (Thundathil et al., 1999). These synchronization systems are promising if a treatment to synchronize ovulation for fixed-time AI would be included.

2.3 Manipulation of follicular development.

2.3.1 Steroid hormones: Progesterone and estradiol.

After progesterone was observed to alter the estrous cycle in cattle by suppressing estrus and preventing ovulation (Christian and Casida, 1948), studies have been conducted to determine the effects of progesterone on the estrus cycle. In addition, different forms and doses of estrogens were investigated to determine effects on estrus and fertility of the treated cattle. Treatments of beef heifers with estrogens resulted in luteal regression; however, estrus and ovulation did not consistently occur following luteolysis (Wiltbank et al., 1961). Later, estradiol was utilized to induce luteolysis in shortened progestin treatments (Wiltbank et al., 1965; Wiltbank and Kasson, 1968). A dose of 5 mg of estradiol valerate was administered on the second day of a 9-day treatment with 400 mg/head/day of dihydroxy-progesterone acetophenonide (DHPA) in

feed. The treatment resulted in 95% of the heifers displaying estrus over a 96-h period and 54% conceived when inseminated at synchronized estrus (Wiltbank and Kasson, 1968). Thereafter, progestin treatments were shortened to 9 days and an injection of estradiol valerate was administered at the beginning of the treatment period to shorten the luteal life span.

Recently, it was observed that the administration of 5 mg estradiol valerate (EV) along with a norgestomet implant (Syncro-Mate-B, SMB) resulted in regression of antral follicles (Bó et al., 1991), and emergence of a new follicular wave at a relatively predictable time thereafter (Bó et al., 1993; Mapletoft et al., 1999). When estradiol was administered in the late static or regressing phase, emergence of the next follicular wave was attributed to the extended suppression of plasma FSH concentrations (Bó et al., 1993). Therefore, the high estrous response and acceptable pregnancy rates in studies by Wiltbank and Kasson (1968) were not only due to the luteolytic effects of EV, but also to the EV-induced follicle turnover.

Estradiol-17β has been shown to suppress follicular growth when given to progestin-treated cattle (reviewed in Bó et al., 1995a). It was also shown to be equally efficacious, regardless of the stage of follicle development at the time of treatment (Bó et al., 1995a). However, when given to norgestomet-implanted beef cattle at different doses (2.5, 5 or 10 mg), the 5 mg E-17β dose resulted in the least variable interval from treatment to follicular wave emergence. Overall, emergence of a new follicular wave occurred 4.3 ± 0.2 days after treatment (range: 3 to 5 days) in 44/47 females (94%; Bó et al., 1995a, 1995b). The im injection of 5 mg of resulted in increased plasma estradiol concentrations reaching peak concentrations 18 h later and decreased circulating FSH

concentrations (Bó et al., 1994). Once estradiol concentrations declined, synchronous FSH release and emergence of a new follicular wave occurred (Bó et al., 1994). The mechanism responsible therefore involves suppression and then rebound release of FSH (Bó et al., 1994) and possibly LH. Emergence of a new follicular wave depends on the resurgence of plasma FSH concentrations that in turn depend on circulating estradiol concentrations.

Different estradiol esters such as estradiol benzoate (EB; Caccia and Bó, 1998, Macmillan, 1994), estradiol valerate (EV; D'Occhio et al., 1996; Niasari-Naslaji et al., 1996; Mapletoft et al., 1999) or E-17β (Kastelic et al., 1996, 1997; Martínez et al., 2000) have been used in combination with different progestin sources in synchronization programs. Progestins have been incorporated with the initial estradiol treatment (for follicular wave synchronization) to prevent an estrogen-induced LH surge (Bó et al., 1994). Highly acceptable pregnancy rates were obtained in the synchronization of follicular wave emergence in estrus synchronization protocols for AI (Kastelic et al., 1996).

2.3.2 Gonadotrophin releasing hormone or luteinizing hormone.

Although GnRH directs the estrous cycle in cattle, there is endogenous control of its secretion. The use of exogenous GnRH offers the possibility of controlling follicular events during the estrous cycle. GnRH has been commercially available since the 1970's and is used widely (Drost and Thatcher, 1992), predominantly for the treatment of follicular cysts in cattle (Kittok et al., 1973). More recently, the effect of GnRH on

follicular dynamics has been investigated. An experiment was designed to study the effect of a single injection of a GnRH-analog (buserelin) on follicular dynamics (Macmillan and Thatcher, 1991). Treatment with GnRH resulted in ovulation of the largest follicle present at the time of treatment, but not in all cattle. It was concluded that these changes could have altered the normal wave patterns of follicular development. In another study, heifers treated with GnRH 32 h before ovulation had a shortened interval from the LH surge to the emergence of the first follicular wave (Bodensteiner et al., 1996). After GnRH treatment, the dominant follicle would ovulate and a new follicular wave would emerge within 2 days after treatment (Twagiramungu et al., 1995). However, administration of GnRH at random stages of the estrous cycle has not always resulted in ovulation (Kastelic and Mapletoft, 1998). Ovulation depended on the stage of development of the dominant follicle (Twagiramungu et al., 1995). The use of GnRH at different and variable stages of the first follicular wave was investigated in two preliminary studies (Prescott et al., 1992; Silcox et al., 1993), but results were inconclusive. In a more recent study (Martínez et al., 1999), the administration of GnRH or LH to heifers at precise stages of the first follicular wave (Days 3, 6, or 9 after spontaneous ovulation) resulted in ovulation in 56% and 78% of heifers, respectively. Follicular wave emergence was only induced within 2 days when ovulation of the dominant follicle occurred (Martínez et al., 1999). With the remaining non-ovulated heifers, a new follicular wave emerged spontaneously within 2 days.

GnRH has not resulted in acceptable synchronization estrus and ovulation when used in synchronization protocols in heifers. This was due to poor synchronization of wave emergence after the first treatment, and by the time PGF treatment was given 6 or

7 days later, the interval to estrus was highly great variable. Furthermore, if spontaneous luteolysis occurred during the period between GnRH and PGF treatment, heifers would express estrus (Roy and Twagiramungu, 1999), since GnRH did not result in ovulation of the dominant follicle. When GnRH treatment was followed by PGF 7 days later, a high number of cattle was observed in estrus over the following 5 days (Thatcher et al., 1993).

2.3.3 Follicular ablation.

Physical methods of follicular ablation, such as cautery, have been used to remove antral follicles (Ko et al., 1991; Adams et al., 1992a, 1993a, 1993b). The effect of follicular puncture and aspiration on the estrous cycle has also been examined. Pieterse et al. (1991) observed no changes in estrous cycle length among animals in which follicles were ablated on Days 3 to 4, 9 to 10 or 15 to 16. However, in another study, follicle-aspirated females had extended cycles compared with non-aspirated controls, presumably due to the time required for growth of a new dominant follicle to an ovulatory size (Stubbings and Walton, 1995). Based on studies of follicular ablation by electrocautery carried out by Adams et al. (1992a, 1993a), ultrasound-guided transvaginal follicle aspiration at random stages of the estrous cycle was used to synchronize follicular wave emergence and to improve the synchrony of ovulation after PGF treatment (Bergfelt et al., 1994). An FSH surge occurred after ablation of all follicles ≥5 mm in the ovaries, resulting in emergence of a new follicular wave within 2 days after aspiration. Treatment with PGF 4 days after ablation resulted in synchronous

ovulation. A greater synchrony of ovulation was obtained when two doses of PGF were given 12 h apart (at 4.0 and 4.5 days after follicular ablation) and GnRH or pLH was administered 24 h later to induce ovulation (Brogliatti et al., 1998). When ultrasound-guided follicular aspiration was used to synchronize follicular wave emergence in CIDR-B-based synchronization programs, synchronous follicular wave emergence occurred within 1.0 ± 0.1 days after ablation, and ovulation occurred 3.0 to 4.5 days after CIDR-B removal, with pregnancy rates to AI after estrus detection of 65% (Martínez et al., 2000). Although follicular ablation offers a great alternative for synchronization of follicular wave emergence and ovulation, this technique may be more appropriate for synchronization of follicular wave emergence in superstimulation protocols; follicle ablation induces a highly synchronized follicular wave emergence but it requires appropriate instrumentation and facilities which makes it less feasible under field conditions. More recently, we have shown that ablation of the two largest follicles in the ovaries were adequate to induce synchronous follicle wave emergence in a superstimulatory program (Baracaldo et al., 2000).

2.4 Control of luteal and follicular dynamics for synchronization of ovulation using estradiol and progesterone.

The use of estradiol and progesterone in estrus synchronization protocols is based on the hypothesis that estradiol treatment will synchronize emergence of a new dominant follicle capable of ovulating after PGF-induced induced luteolysis, with a highly acceptable pregnancy rates following AI. Bó et al. (1995a) showed that the

combination of estradiol-17β (E-17β) and progesterone synchronized follicular wave emergence in CIDR-B-treated beef cattle, resulting in 75% of heifers ovulating between 72 and 84 h following CIDR-B removal and PGF administration. In another similar study, the mean interval from treatment to wave emergence was 3.4 ± 0.1 days, and following CIDR-B removal, all heifers ovulated, with a pregnancy rate of 80.0% to AI after estrus detection (Martínez et al., 2000). This treatment protocol was considered to have great potential in ovulation synchronization programs.

Estradiol and progesterone in combination have been used in other progestin-based protocols. It has been shown that an injection of progesterone (100 mg) or a CIDR-B for 24 h on Day 10 of a 17-day norgestomet protocol in *Bos indicus* heifers synchronized follicular wave emergence and ovulation (Cavalieri et al., 1998). Treatment with 100 mg progesterone provided a shorter interval from implant removal to estrus (38.4 ± 2.6 h) than 200 mg progesterone (61.5 ± 3.9 h; Cavalieri et al., 1998). The combination of estradiol and progesterone was effective in synchronizing follicular wave emergence but the dose of hormone used may affect results.

In our laboratory, 5 mg of E-17β and 100 mg progesterone have been given on Day 0 of a 7-day MGA program with PGF treatment on the last day of MGA feeding. The synchronized pregnancy rate in heifers without a CL (apparently not cyclic) was higher in the group treated with estradiol and progesterone than that in the non-treated Control group (52% vs 20%, respectively; Kastelic et al., 1997). The low pregnancy rates in the Control group receiving MGA without follicular wave synchronization can be a direct result of the development of persistent follicles that ovulated aged oocytes after MGA withdrawal.

High accuracy in the time of ovulation becomes important for fixed-time AI. If a follicular wave is synchronous, it will be possible to predict the day to induce ovulation with estradiol (Lammoglia et al., 1998), LH (Brogliatti et al., 1998) or GnRH (Pursley et al., 1995). Estradiol has been used to synchronize estrus in PGF-based estrus synchronization protocols (Dailey et al., 1983; Peters et al., 1977; Welch et al., 1975) and induced estrus, LH release and ovulation in CIDR-B-treated cattle (Lammoglia et al., 1998).

2.5 Development of GnRH-based regimens for synchronization of ovulation.

The first attempts of using GnRH in an estrus synchronization program involved GnRH treatment to synchronize follicular wave emergence, followed by PGF given 6 (Twagiramungu et al., 1992) or 7 days later (Wolfenson et al., 1994) to cause luteolysis of the new-formed and/or old CL.

A 10-day estrus synchronization program with a 6-day interval between GnRH and PGF treatments, followed by a second GnRH treatment 48 h after PGF, was proposed for improving the precision of estrus without reducing fertility (Twagiramungu et al., 1995). In another study, a second GnRH treatment after PGF injection improved the precision of estrus, and permitted fixed-time insemination without adversely affecting conception rate (Pursley et al., 1995). This method consisted of an injection of GnRH followed by PGF 7 days later, a second GnRH injection 48 hours after PGF treatment, and AI from 0 to 24 hours later. Cows receiving the second GnRH treatment had a higher rate of ovulation than Control cows receiving only saline (97% vs. 77%,

respectively). The first injection of GnRH apparently induced LH release and ovulation of the dominant follicle present at that time in 90% of cows and 50% of heifers, resulting in the emergence of a new follicular wave within 2 days after treatment. The administration of PGF 7 days after treatment apparently induced the regression of the original and/or the induced CL. The second GnRH injection was given to induce LH release and synchronous ovulation of the new, GnRH-induced, dominant follicle.

This program called "Ovsynch" (Seguin, 1997) has been widely used on dairy farms in North America over the last few years (Wiltbank, 1997). It seems to be especially efficacious in dairy farms in which cows in estrus are not effectively detected (Wiltbank, 1997). The use of an estrus synchronization program with GnRH followed by PGF treatment 6 days later in beef cows resulted in pregnancy rate similar to that of cows bred to a spontaneous estrus (Roy and Twagiramungu, 1996), suggesting that this program could be utilized in beef herds. The use of a second GnRH treatment given at the time of AI (48 h after PGF) in cows seemed to facilitate fixed-time AI (Geary et al., 2001). However, the Ovsynch program has not been successfully used in dairy heifers (Pursley et al., 1995).

It has been reported that many heifers were detected in estrus between the first injection of GnRH and PGF. Many factors can be responsible for this, such as lack of response to the first GnRH injection. When GnRH treatment was given to beef heifers on Day 3, 6, or 9 of the first follicular wave, 56% ovulated and therefore, started a new follicular wave within 2 days (Martínez et al., 1999). It was proposed that by reducing the interval from the first GnRH to PGF treatment would reduce the number of heifers detected in estrus during that period of time (i.e., 6 days a instead of 7 days). However,

it was found that 9% of heifers showed estrus during a 6-day interval while 12% of heifers were detected in estrus during 7-day interval (Roy and Twagiramungu, 1999). The use of a progestin-releasing device or MGA feeding may be a alternative to suppress estrus display during an Ovsynch program for fixed-time AI due to the increase in plasma progesterone that is released.

A synchronization protocol consisting of a 6-day CIDR-B insertion with GnRH given at insertion and PGF treatment given and CIDR-B removal resulted in acceptable pregnancy rates (65%) to AI after estrus detection (75%; Martínez et al., 2000). However, the asynchrony of follicular growth at the time of the second GnRH treatment has not been reduced sufficiently to obtain acceptable pregnancy rates to fixed-time AI in heifers.

2.6 Problems associated with estrus or ovulation synchronization and artificial insemination.

The gold standard in determining that a cow is in estrus has been the visual observation of that cow "standing" while being mounted by a herd mate. Effective detection of estrus is important for success and profitability of any AI program. Most of the Canadian dairy herds (70%) that apply AI demonstrate that achievements of good results are feasible, but heat detection is variable and often inadequate. Detection of estrus is the most limiting factor in optimizing reproductive efficiency in dairy herds utilizing AI. Detection efficiency is equal to or less than 50% in most herds (Nebel, 2000).

Estrus detection affects fertility criteria of any herd and is the number one factor responsible reproductive performance. The first cause of non-fecundity in a herd is most often the difficulty to detect estrus and this inefficiency can be partially due to biological characteristics of the cow (i.e., short estrus, increased milk production in dairy cattle, lameness, etc.), and to breeding practices (i.e., time spent detecting estrus, criteria used by the breeder, herd size, etc.). Basically, this can be summarized in two components: the animal (physiology) and the breeder (observation and experience).

Estrus synchronization in cattle has disadvantages that must be considered. Twice-daily estrus detection allows for acceptable conception rates when AI is performed 8 to 12 hours after onset of estrus (Foote, 1975). However, estrus detection can be tedious, inaccurate and, in some cases, very ineffective. Inaccurate detection of estrus can be considered as a result of failure by herd personnel to accurately identify females in estrus (Foote, 1975). Other studies have determined that between 5% and 30% of inseminations were performed when females were not in estrus (Senger et al., 1988), and that 19% of inseminations have been carried out when progesterone concentrations were high (during the estrous cycle or in pregnant cows; Sturman et al., 2000). The use of computerized systems has simplified the task of estrus detection (Walker et al., 1996). Even though synchronization programs facilitate all animals to come into estrus in a relatively short period of time, moving and sorting individuals or groups of heifers or cows with their calves into the working facilities is required. These tasks are often not practical or feasible for many beef producers.

Undetected or falsely detected estrus results in inseminations being performed at inappropriate times and in an increased calving interval, leading to losses in milk and calf production. It was reported that the insemination of pregnant animals caused 17% of embryonic mortality or abortions. Conception rates resulting from insemination of cows with milk progesterone greater than 10 ng/ml were significantly lower than inseminations at lesser concentrations of progesterone (<2 mg/ml; Sturman et al., 2000).

Effective estrus detection in cattle ensures a great part of the success of AI. It has been emphasized that primary considerations for an "ideal" system for estrus detection are continuous surveillance, accurate and automatic identification of the female in estrus, operation for the productive lifetime of the animal, minimal or no labor requirements and high accuracy to identify behavioural events that correlate with ovulation (Senger, 1994). Among the present aids for estrus detection, the pressure-sensitive electronic mount detection systems seem to be the only alternative that include most features for the "ideal" method of estrus detection, except cost and maintenance. These are two reasons that avoided the widespread use of this electronic method.

Finally, if estrus detection may be eliminated, and high pregnancy rates are obtained after a fixed-time AI program, the use of AI in beef herds will be increased. Hence, it is critical that the process of taking animals to the facilities and the time spent, and all the associated labor must be reduced in order to apply the AI technology in a beef operation. Insemination by appointment without the need for estrus detection, becomes an important tool for the expansion of the use of AI in beef herds.

2.7 Fixed-time artificial insemination.

Because inaccurate detection of estrus can be translated into economic losses, aids for estrus detection and estrus synchronization can be considered at least partial solutions (Larson and Ball, 1992). The control of the estrous cycle will increase the number of females inseminated at the beginning of the breeding season and focus the efforts to observe cows at an expected time. However, the critical part of this puzzle has not yet been resolved, i.e., the need for accurate detection and the inaccuracy of estrus detection. The successful use of protocols that would allow for insemination by appointment (referred to as fixed-time AI) could completely eliminate the need for estrus detection.

Fixed-time AI has not been completely successful over the past three decades. Although conception rate following AI at 72 and 96 h after the second dose of PGF was similar to that following AI after estrus detection (Lauderdale, 1972), fertility after a single fixed-time AI in PGF-based protocols is low. More recently, pregnancy rates of 35 % have been reported in programs of a single AI after two doses of PGF 14 days apart, (Seguin et al., 1999). Therefore, programs that consisted of two doses of PGF followed by two inseminations have not been implemented in beef or dairy herds (Peters, 1986). In addition, the double amount of semen would increase the cost of the AI considerably. The combinations of progestins with PGF or progestins with EB as a luteolytic agent for timed AI have also been used, but again pregnancy rates have been poor (Larson and Ball, 1992). Results obtained from pioneer studies demonstrate that fertility has been disappointingly low when the estrous cycle is controlled only through the induction of luteal regression (without manipulation of follicles).

The development of modern synchronization programs that include the control of both luteal function and follicular dynamics with predictable intervals to ovulation will facilitate fixed-time artificial insemination.

3.0 GENERAL HYPOTHESIS

Synchronization of ovarian follicular waves and ovulation will facilitate fixed-time artificial insemination and result in pregnancy rates comparable to insemination after detected estrus.

3.1 GENERAL OBJECTIVE

The overall objective of this research was to develop new synchronization protocols that facilitate fixed-time artificial insemination. Secondary objectives were to improve fertility in some of the current ovulation synchronization programs and to compare different programs involving manipulation of CL and follicular growth for synchronization of follicular wave emergence and ovulation.

4.0 GENERAL MATERIALS AND METHODS

4.1 Animals: The cows and heifers used in the experiments described in Chapters 5.0 and 6.0 of this thesis were housed outdoors in feedlot pens at the Goodale Research Farm, University of Saskatchewan, and fed barley silage, with free access to water. The cows used in Experiment 1 of Chapter 6.0 were kept on pasture consisting of oats and native grass with free access to water during the winter in Córdoba, Argentina (31° 24' S, 64° 11' W, 400 meters above sea level). The cattle used in field experiments (Chapters 7.0 to 11.0) were housed in feedlot pens (heifers in Chapter 7.0, 8.0.). However, the cows used in Experiment 2 of Chapter 7.0 were kept on pasture of native grass in southern Alberta, and heifers in Experiment 3 of Chapter 10.0 were kept on pasture during the spring in Córdoba.

4.2 Body condition score was evaluated based on a scale from 1 (lean) to 5 (obese; Mackey et al., 2000).

4.3 Breeding season: All field experiments involving fixed-time AI were carried out during the spring and beginning of the summer (April 15 to July 1).

4.4 CIDR-B device preparation: Devices were kept in a mild iodine solution (0.1-0.5%; Betadine solution 10%; Purdue Frederick Inc, Pickering, ON, Canada) immediately prior to use. The applicator was always washed and then, kept in a strong iodine solution (5%) before use in each female. The CIDR-B was introduced into the applicator by folding its arms (changing from T- to I-shape) and then, inserted into the anterior vagina by pushing forward the plunger of the applicator, allowing the device to expand its arms and recovering the original T-shape.

4.5 MGA preparation: In the MGA-based protocols, MGA was obtained from a commercial source as a premix at a concentration of 220 mg/kg and then, it was mixed with the remaining ration to be fed. The size of the feeding bunk was sufficient to allow animals to consume equal amount of ration at the same time, so that the MGA intake could be evenly distributed.

4.6 Hormone preparation and injections: Steroid hormones were diluted in benzyl alcohol (10% of the final volume), stirred for 15 minutes and then canola oil was added to the final volume. The total dose of steroid hormones administered was contained in 2 mL of canola oil in all experiments throughout the thesis. The commercial preparations of GnRH and prostaglandin were injected at the dose recommended by the manufacturer, 100 μg gonadorelin and 500 μg cloprostenol, respectively. Porcine LH was administered at one half (12.5 mg) of the recommended dose (25 mg; Experiment 3, Chapter 10.0). All injections were given intramuscularly in the gluteal region.

4.7 Estrus detection: In all experiments, estrus detection was performed by one person at 12 h intervals (e.g.; 7:00 am and 7:00 pm) for at least 30 minutes, except in Experiment 4 in Chapter 10.0 and the two experiments of Chapter 11.0 in which females were observed by two or more persons. Only those animals, which were standing to be mounted for more than 4 seconds were considered to be in standing estrus.

4.8 Animal care: The University of Saskatchewan Animal Care Committee has approved the protocols (No. 19970080) for these experiments.

4.9 Statistical packages: Almost all statistical analyses were conducted using SAS (SAS User's Guide, 1990; SAS Institute Inc., Cary, NC, USA). One-way analysis of variance followed by LSD test or non-parametric tests (Mann-Whitney two-sample rank sum test; Kruskal-Wallis test, Bartlett's test for homogeneity of variances; Norman and Streiner, 2000) were performed by using Statistix (Statistix Student Version, version 2.0, Analytical Software, Tallahassee, Florida, USA). Logistic regression in Chapter 9.0 was performed by using SPSS (SPSS version 10.05, 1999, SPSS Inc., Chicago, IL, USA).

4.10 Probabilities: A probability of 5% ($P < 0.05$) was used to determine that a difference was significant, and probabilities between 5 and 10% ($P > 0.05$ to $P < 0.10$) were used as an indication that a difference approached significance (e.g.; tendency to be significantly different). However, actual probability levels obtained from the statistical analysis (i.e., $P < 0.0001$, $P = 0.4$, etc.) have been reported in all chapters of this thesis.

5.0 EFFECTS OF STEROID HORMONES ON GONADOTROPHIN RELEASE IN OVARIECTOMIZED COWS TREATED WITH A CONTROLLED INTERNAL DRUG RELEASE (CIDR-B) DEVICE.

5.1 Abstract

A series of experiments were conducted to evaluate the effects of estradiol and progesterone on gonadotrophin release in ovariectomized cows. In Experiment 1, ovariectomized cows (n = 16) received a used CIDR-B device and 5 mg estradiol-17β (E-17β) on Day 0 and were randomly allocated to four treatment groups to receive 0, 25, 50, or 100 mg of progesterone. The CIDR-B devices were removed 7 d later. In all groups, plasma progesterone concentrations were elevated by 24 h after insertion of the CIDR-B devices ($P < 0.0001$). Mean plasma LH concentrations were similar among groups ($P = 0.7$); mean plasma LH decreased ($P < 0.0007$) by 36 h after treatments began, remained low ($P = 0.013$) until 72 h, and returned to pretreatment concentrations 12 h later (i.e., 84 h after treatment). There was no effect of progesterone treatment on plasma FSH concentrations ($P = 0.5$). In Experiment 2, ovariectomized cows (n = 17) were randomly assigned to receive a new CIDR-B device or nothing on Day 0 (beginning of the experiment). On Day 5, half of each group received an injection of either 5 mg of E-17β or 5 mg of E-17β + 100 mg of progesterone. The CIDR-B devices were removed on Day 10. Plasma progesterone concentrations increased ($P < 0.0001$) by 24 h after CIDR-B insertion. There was an effect of time ($P < 0.0001$) on plasma LH and FSH concentrations. Plasma LH concentrations declined ($P < 0.05$) by 6 h after

treatment on Day 5 and LH surges occurred between 18 h and 24 h in groups receiving E-17β without or with progesterone, respectively. There was a decrease ($P < 0.0001$) in plasma FSH concentrations by 6 h and resurgence by 48 h after estradiol treatment. In Experiment 3, ovariectomized cows (n = 16) received a used CIDR-B device on Day 0 (beginning of the experiment) and were allocated randomly into three treatment groups to receive: 5 mg of E-17β on Day 0 (Group E0), 5 mg of E-17β on Day 1 (Group E1), or 5 mg of E-17β + 100 mg of progesterone on Day 0 (Group EP). The CIDR-B devices were removed on Day 7 and an injection of 1 mg estradiol benzoate (EB) was given to all cows on Day 8 (24 h later). There was an effect of time and treatment-by-time interaction ($P < 0.0001$) on plasma progesterone, estradiol, LH and FSH concentrations. Administration of 100 mg of progesterone at CIDR-B insertion resulted in an increase ($P < 0.0001$) of 2.0 ng/mL progesterone over CIDR-B alone. The use of a CIDR-B device alone suppressed LH for 6 h. Estradiol-17β, administered with or without progesterone, induced an LH surge within 24 h and suppressed plasma FSH for approximately 48 h. Plasma progesterone concentrations declined to baseline by 12 h after CIDR-B removal. For the second part of this experiment, the injection of 1 mg EB resulted in transient LH and FSH suppression ($P < 0.02$) by 6 h comparable to that of 5 mg of E-17β at the time of CIDR-B insertion, followed by an LH surge at 24 h. In Experiment 4, ovariectomized cows (n = 16) received a used CIDR-B device on Day 0 (beginning of the experiment) and were allocated randomly to 3 treatment groups to receive 3 different forms of estradiol: 5 mg of E-17β, EB or estradiol valerate (EV) plus 100 mg of progesterone in 2 mL canola oil. The CIDR-B devices were removed on Day 7. There was an effect of time and treatment-by-time interaction ($P < 0.0001$) on plasma

estradiol and FSH concentrations. Plasma estradiol concentrations increased and reached a higher peak ($P < 0.01$) more rapidly after E-17β treatment than following treatment with EB or EV. Plasma estradiol concentrations were at baseline by 36 h in E-17β-treated cows and by 96 h in EB- and EV-treated cows. Plasma FSH concentrations decreased ($P < 0.0001$) after estradiol treatment in all groups, reached a nadir at 24 h and increased by 60 h in all groups, but concentrations remained higher ($P < 0.02$) in E-17β-treated than in EB- or EV-treated cows. In summary, the use of a new CIDR-B device increased plasma progesterone to near luteal concentrations, but for only 2 days. Administration of 100 mg progesterone to ovariectomized cows at CIDR-B insertion increased plasma progesterone approximately 2.0 ng/mL compared to those that received only a CIDR-B device. Progesterone did not affect plasma FSH concentrations. Administration of E-17β, with or without progesterone, caused a decrease in LH concentration followed by an LH surge within 24 h after treatment. Estradiol treatment resulted in FSH suppression with concentrations and length of suppression dependent on the estradiol formulation. Gonadotrophin response to the administration of 1 mg of EB after CIDR-B removal was equivalent to that induced by 5 mg of E-17β at CIDR-B insertion.

5.2 Introduction

Estradiol and progesterone have been used in estrus synchronization programs. Both hormones are usually administered at the time of insertion of a progestin-releasing device to synchronize follicular wave emergence. Synchronous ovulation is usually induced by giving a small dose of estradiol (e.g., 1.0 mg) after progestin removal (Lammoglia et al., 1998).

Estradiol affects follicular wave dynamics (Bó et al., 1991, 1993, 1994, 1995a). When estradiol-17β (E-17β) was given to heifers at different stages of the first follicular wave, emergence of a new follicular wave occurred, on average, 4.3 days later (Bó et al., 1995b). However, E-17β caused regression of early stage dominant follicles only in heifers that had previously received a progestin implant (Bó et al., 1994). Plasma FSH concentrations decreased by 6 h and increased between 30 and 72 h after estradiol treatment in norgestomet-implanted heifers (Bó et al., 1994).

Different estradiol esters may be expected to have different effects on FSH secretion. Estradiol valerate (EV) has a long life in the circulation and a prolonged suppressive effect on FSH and ovarian follicular growth (Bó et al., 1991, 1993). When 5 mg EV was given to heifers on Day 1 after ovulation, early emergence of the second follicular wave occurred, while treatment on Days 3 or 6 delayed follicular wave emergence as compared to Control heifers (Bó et al, 1993). Estradiol benzoate (EB) also has a long circulating life; treatment with 5 mg EB resulted in the emergence of a follicular wave, on average, 1 day later than in cows given 5 mg E-17β (Bó et al., 1996).

However, a contemporaneous comparison of the effects of E-17β, EB and EV on gonadotrophin concentrations has not been made.

The administration of E-17β to ovariectomized cattle caused an initial decrease in plasma LH concentrations followed by an LH surge, approximately 12 to 24 h later (Short et al., 1973; Hausler and Malven, 1976; Schoenemann et al., 1985). A norgestomet implant or a series of progesterone injections also decreased LH pulsatility and blocked the estradiol-induced LH surge (Schoenemann et al., 1985; Bolt et al., 1990). When 10 mg E-17β was given (alone or in combination with norgestomet) to ovariectomized cattle, plasma FSH concentrations were depressed for 64 h (Bolt et al., 1990). Therefore, estradiol would appear to have a differential effect on LH and FSH and the effect on LH was dependent on the presence or absence of progesterone.

Progesterone-releasing devices (i.e., CIDR-B, PRID) have been used widely to synchronize estrus and ovulation. However, these devices have also been used to create models of follicular persistence (Savio et al., 1993). In intact cows, a PRID altered LH secretion, leading to an increase in LH pulsatility and development of persistent follicles (Roberson et al., 1989; Kojima et al., 1992). However, when two devices were used, the resulting elevated plasma progesterone concentrations caused a decrease in mean LH concentrations and pulsatility (Roberson et al., 1989; Kojima et al., 1992) and prevented formation of persistent follicles. We hypothesized that the development of persistent follicles could be prevented in CIDR-B-treated cattle by administering estradiol at the time of CIDR-B insertion, which would result in suppression of the present dominant follicle followed by the emergence of a new follicular wave (Bó et al., 1995a; Martínez et al., 2000). This combination induced a suppression of the dominant follicle followed

by a resurgence of FSH and the subsequent emergence of a new follicular wave. The effect of various doses of EB (along with CIDR-B devices) has been studied (O'Rourke et al., 2000). However, as different estradiol esters have been used in the manipulation of follicular wave dynamics for estrus synchronization, we considered that a comparison of their effects on gonadotrophins in ovariectomized cattle would be important.

A series of experiments were designed to determine the effects of estrogen and progesterone treatments on circulating concentrations of gonadotrophins in ovariectomized cows. The objective of the first experiment was to determine the dose of progesterone, given concurrently with a standard dose of E-17β, capable of affecting LH release in CIDR-B-treated, ovariectomized cows. The second experiment was designed to determine the effect of a CIDR-B device on basal circulating gonadotrophin concentrations in ovariectomized cows, and the effect of E-17β (alone or with progesterone) on gonadotrophin release. The third experiment was designed to determine the effect of E-17β on gonadotrophin concentrations when administered with low or high plasma progesterone concentrations and to determine timing of LH release after CIDR-B removal and treatment with EB. The fourth experiment was designed to determine: 1) the circulating life-span of three different estradiol forms; 2) gonadotrophin profiles after treatment with three different estradiol forms administered with progesterone; and 3) the period of inhibition of FSH-release after administration of the three different estradiol forms in progesterone-treated, ovariectomized cows.

5.3 Materials and Methods

5.3.1 Animals

Beef cows (n = 17), which had been ovariectomized 11 to 12 months earlier, were used in these experiments. Cows were housed outdoors in feedlot pens at the Goodale Research Farm, University of Saskatchewan and fed barley silage, with free access to water.

5.3.2 Experiment 1

On Day 0 (beginning of the experiment), cows (n=16) received a once-used CIDR-B (Vetrepharm Canada Inc, Belleville, ON, Canada) device and 5 mg of E-17β (Sigma-Aldrich Canada Ltd, Oakville, ON, Canada) in 2 mL canola oil and were randomly allocated to four groups treated 0, 25, 50 or 100 mg of progesterone (Sigma-Aldrich Canada Ltd) in 2 mL canola oil. The CIDR-B devices were removed 7 d later. Blood samples were taken every 12 h from CIDR-B insertion to the end of the experiment to measure progesterone, estradiol, FSH and LH (Figure 5.1).

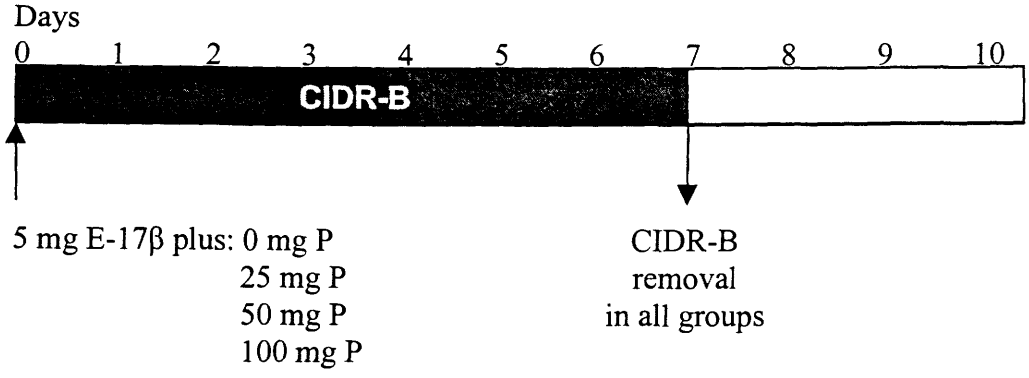


Figure 5.1 Treatment schedule of ovariectomized beef cows (n = 16) receiving a once-used CIDR-B device on Day 0 (beginning of the experiment) and concurrently injected i.m. with 5 mg E-17β plus 0, 25, 50 or 100 mg progesterone (P) im. The CIDR-B devices were removed on Day 7. Blood samples were taken every 12 h from CIDR-B insertion to the end of the experiment to measure progesterone, estradiol, FSH and LH.

5.3.3 Experiment 2

Seventeen cows were assigned randomly to receive a new CIDR-B device or nothing on Day 0 (beginning of the experiment). On Day 5, half of each group received an i.m. injection of 5 mg E-17β or 5 mg E-17β plus 100 mg progesterone in 2 mL canola oil. The CIDR-B devices were removed on Day 10. Blood samples were collected every 24 h from CIDR-B insertion to Day 10 for progesterone measurement, every 6 h for 48 h after E-17β; and every 12 h to the end of the experiment on Day 10 for progesterone, LH, and FSH analysis (Figure 5.6).

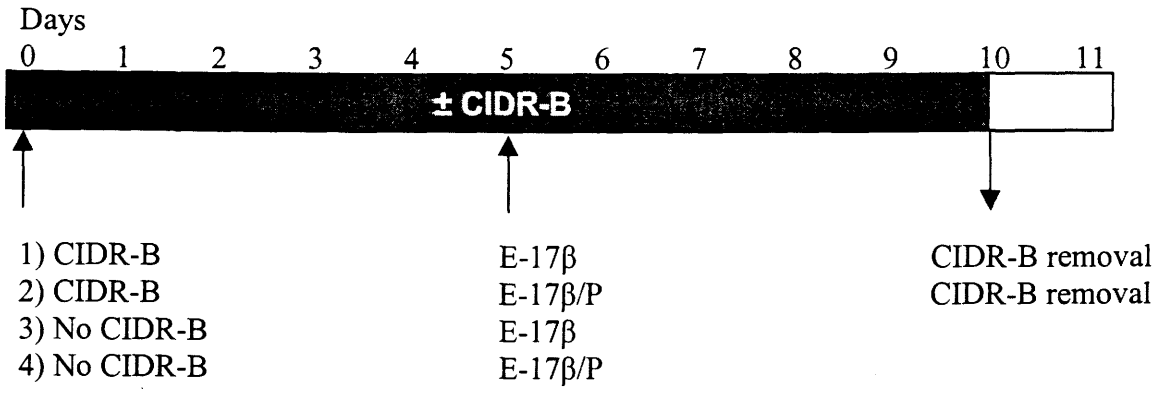


Figure 5.2 Treatment schedule of ovariectomized beef cows (n = 17) after insertion of a progesterone-releasing CIDR-B device or not (Day 0) followed by an i.m. injection of E-17β (E-17β) or E-17β plus progesterone (E-17β/P) on Day 5. The CIDR-B devices were removed on Day 10 (as indicated by arrows). Blood samples were collected every 24 h from CIDR-B insertion to Day 10 for progesterone measurement; every 6 h for 48 h after E-17β and progesterone treatment and, every 12 h to the end of the experiment on Day 10 for estradiol, LH and FSH analysis.

5.3.3 Experiment 3

Ovariectomized beef cows (n = 16) received a once-used CIDR-B device on Day 0 (beginning of the experiment; 2 d after completion of Experiment 1). Cows were allocated randomly to three groups treated i.m. with 5 mg E-17β on Day 0 (Group E0), 5 mg E-17β on Day 1 (Group E1), or 5 mg E-17β plus 100 mg P on Day 0 (Group EP). The CIDR-B devices were removed on Day 7 and all cows were given 1 mg i.m. of EB (Sigma-Aldrich) in 2 mL canola oil on Day 8. Blood samples were taken every 6 h for a period of 72 h after CIDR-B insertion, then every 12 h until 24 h after CIDR-B removal (Day 8), and every 6 h for 48 h thereafter for progesterone, estradiol, LH, and FSH analysis (Figure 5.3).

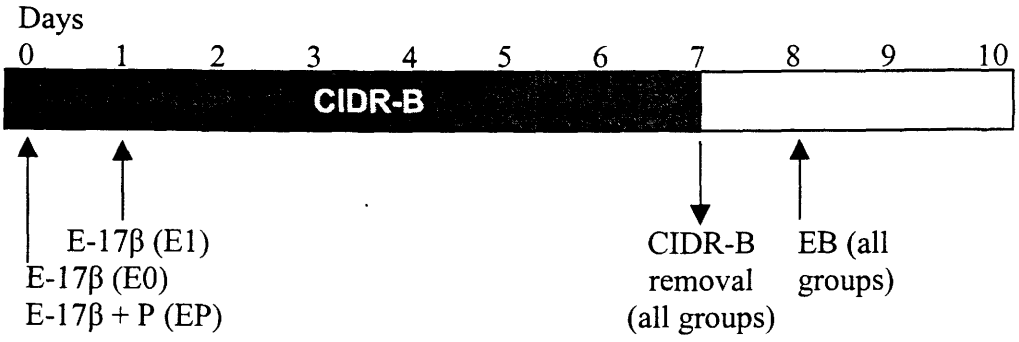


Figure 5.3 Treatment schedule of ovariectomized beef cows (n = 16) treated with a once-used CIDR-B device on Day 0 along with the administration of 5 mg E-17β (E-17β) on Day 1 (E1) or on Day 0 (E0), or 5 mg E-17β and 100 mg progesterone (P) on Day 0 (EP). The CIDR-B devices were removed on Day 7 (as indicated by arrows), followed 24 h later by an injection of 1 mg of EB (Day 8). Blood samples were taken every 6 h for a period of 72 h after CIDR-B insertion, then every 12 h until 24 h after CIDR-B removal, and then, every 6 h for 48 h.

5.3.4 Experiment 4

Sixteen ovariectomized beef cows received a once-used CIDR-B device on Day 0 (beginning of the experiment) and were allocated randomly to three groups treated with 100 mg of progesterone plus 5 mg of one of 3 different estradiol esters: E-17β, EB or EV in 2 mL canola oil. The CIDR-B devices were removed on Day 7. Blood samples were collected every 6 h from Cay 0 to Day 3 and every 12 h thereafter to measure progesterone, estradiol, FSH and LH (Figure 5.4).

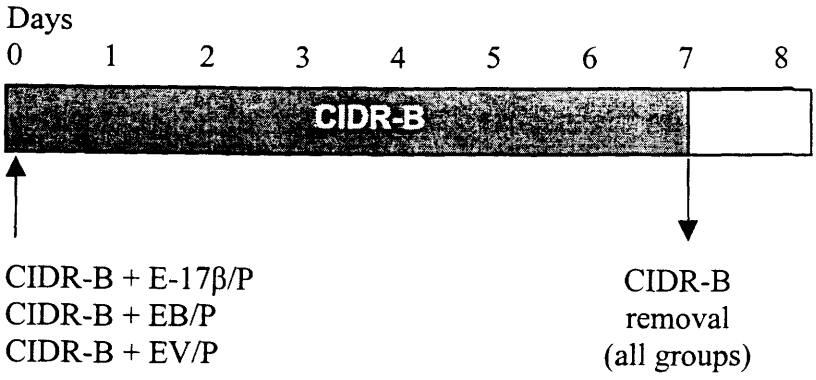


Figure 5.4 Treatment schedule of ovariectomized beef cows treated with a once-used CIDR-B device on Day 0 (beginning of the experiment) and randomly assigned to three groups to receive 100 mg progesterone plus 5 mg E-17β, EB or EV i.m. in 2 mL canola oil. The CIDR-B devices were removed on Day 7. Blood samples were collected every 6 h from Days 0 to 3 and every 12 h thereafter to measure progesterone, estradiol, FSH and LH.

Blood samples for estradiol, progesterone, LH and FSH radioimmunoassay were collected in heparinized tubes, kept at approximately 4°C and centrifuged within 4 h after collection; plasma was stored frozen at –20°C until assayed.

The progesterone assay for Experiments 1, 3 and 4 were carried out using a solid-phase enzyme-linked immunoassay as described by Del Vecchio et al (1995). Progesterone was extracted with 2.5 mL ether added to 250 μL aliquots of plasma. The intra- and inter-assay coefficients of variation were 5.4 and 10.6 and the sensitivity was 4.0 pg/tube. The progesterone and estradiol assays for Experiment 2 were conducted as described by Rawlings et al. (1984). Progesterone was extracted with 3 mL of hexane added to 200 μL aliquots of plasma. The sensitivity of the progesterone radioimmunoassay was 0.1 ng/mL. Intra-assay coefficient of variation was 9.2% and

4.0% for reference sera with mean levels of 1.8 and 13.4 ng/mL, respectively. Estradiol was extracted with ethyl ether. The sensitivity of the assay was 0.5 pg/mL and the intra-assay coefficient of variation was 5.3% and 9.1% for reference sera with mean levels of 31.7 and 208.6 pg/mL, respectively.

Plasma LH concentrations were determined by double antibody radioimmunoassay (Honaramooz et al., 2000) and are expressed in terms of NIDDK-bLH4. The sensitivity of the assay was 0.06 ng/mL, assessed as the lowest concentration of LH capable of displacing labeled LH from the antibody. Intra-assay coefficient of variation was 7.9% and 5.5% for reference sera with mean LH concentrations of 0.4 and 1.0 ng/mL, respectively. Plasma concentrations of FSH were determined using a liquid-phase antibody radioimmunoassay (Rawlings et al., 1984; Honaramooz et al., 1999). The first antibody used was NIDDK anti-oFSH-1 and FSH concentrations are expressed in terms of USDA-bFSH-1. The sensitivity of the assay was 0.13 ng/mL. Intra-assay coefficient of variation was 6.8% and 6.8% for reference sera with mean FSH concentrations of 1.7 and 3.6 ng/mL, respectively.

Time-series hormone data were analyzed using the GLM procedure with repeated measures of (main effects of treatment, time and the treatment-by-time interaction; SAS User's Guide, 1990). Main effects of treatment and time were compared by a protected LSD test. Mean and standard error of the mean (SEM) were used to describe hormone concentrations at different times. One-way analysis of variance was used to compare the interval from treatment to LH or FSH surges (statistically significant increases of circulating hormone concentrations over 1 ng/mL). In Experiment 2, plasma progesterone concentrations were separately analyzed from

Days 0 to 5 and from Days 5 to 10. In Experiment 2, the Mann-Whitney two-sample rank sum test was used to compare the interval from estradiol treatment to LH peak concentrations in those groups receiving an injection of estradiol (alone or in combination with progesterone). In Experiment 3, data for the first 48 h after estradiol treatment were normalized to E-17β treatment and later analyzed for the effects of treatment, time, and treatment-by-time interaction on plasma FSH and LH concentrations. Although probability of 5% ($P<0.05$) was taken as the level of significance in all analyses, probability levels obtained in the statistical tests (i.e., $P<0.0001$, $P=0.4$, etc.) are reported.

The protocols for these experiments were approved by University of Saskatchewan Animal Care Committee.

5.4 Results

5.4.1 Experiment 1

Progesterone. There was a tendency for an effect of treatment ($P = 0.10$), and there was an effect of time ($P < 0.0001$) and treatment-by-time interaction ($P < 0.0002$) on plasma progesterone concentrations. Mean plasma progesterone concentrations were higher in the groups treated with 50 mg ($P = 0.05$) or 100 mg ($P = 0.02$) of progesterone than in the group treated with 0 mg of progesterone. Maximum concentrations were reached 24 h after treatment ($P = 0.0025$) and were highest ($P < 0.001$) in the group treated with 100 mg (6.2 ± 0.4 ng/mL), intermediate in the groups treated with 25 mg (3.2 ± 0.36 ng/mL)

and 50 mg (3.9 ± 0.4 ng/mL) of progesterone, and the lowest ($P < 0.001$) in the group treated with 0 mg progesterone (2.4 ± 0.4 ng/mL). By 48 h after treatment, plasma progesterone concentrations were attributed only to the CIDR-B devices in all groups ($P>0.4$; Figure 5.5).

Luteinizing hormone. There was no effect of treatment ($P = 0.7$) or treatment-by-time interaction ($P = 0.7$) on plasma LH concentrations; however, there was an effect of time ($P = 0.0001$). In all treatment groups, mean (± SEM) LH concentrations tended to increase ($P = 0.08$) from pretreatment levels (1.4 ± 0.2 ng/mL) by 12 h after estradiol and progesterone treatment (1.9 ± 0.2 ng/mL). Then, LH concentrations decreased ($P < 0.007$) to 0.4 ± 0.2 ng/mL by 36 h after treatment, and remained low until 72 h, and finally increased ($P = 0.01$) to pretreatment concentrations by 84 h, where it remained for the remainder of the sampling period (1 d after CIDR-B removal; Figure 5.5).

Follicle stimulating hormone. There was no effect of treatment ($P = 0.5$) or treatment-by-time interaction ($P = 0.9$), however, there was an effect of time ($P = 0.0001$) on plasma FSH concentrations. FSH concentrations decreased ($P < 0.0001$) from 3.3 ± 0.2 ng/mL at the time of treatment to 2.2 ± 0.2 ng/mL and 1.5 ± 0.2 ng/mL at 12 h and 24 h after estradiol treatment, respectively. Plasma FSH concentrations remained low until 48 h after treatment. Mean (± SEM) plasma FSH concentrations increased ($P < 0.01$) to 3.5 ± 0.2 ng/mL by 60 h after treatment (Figure 5.5).

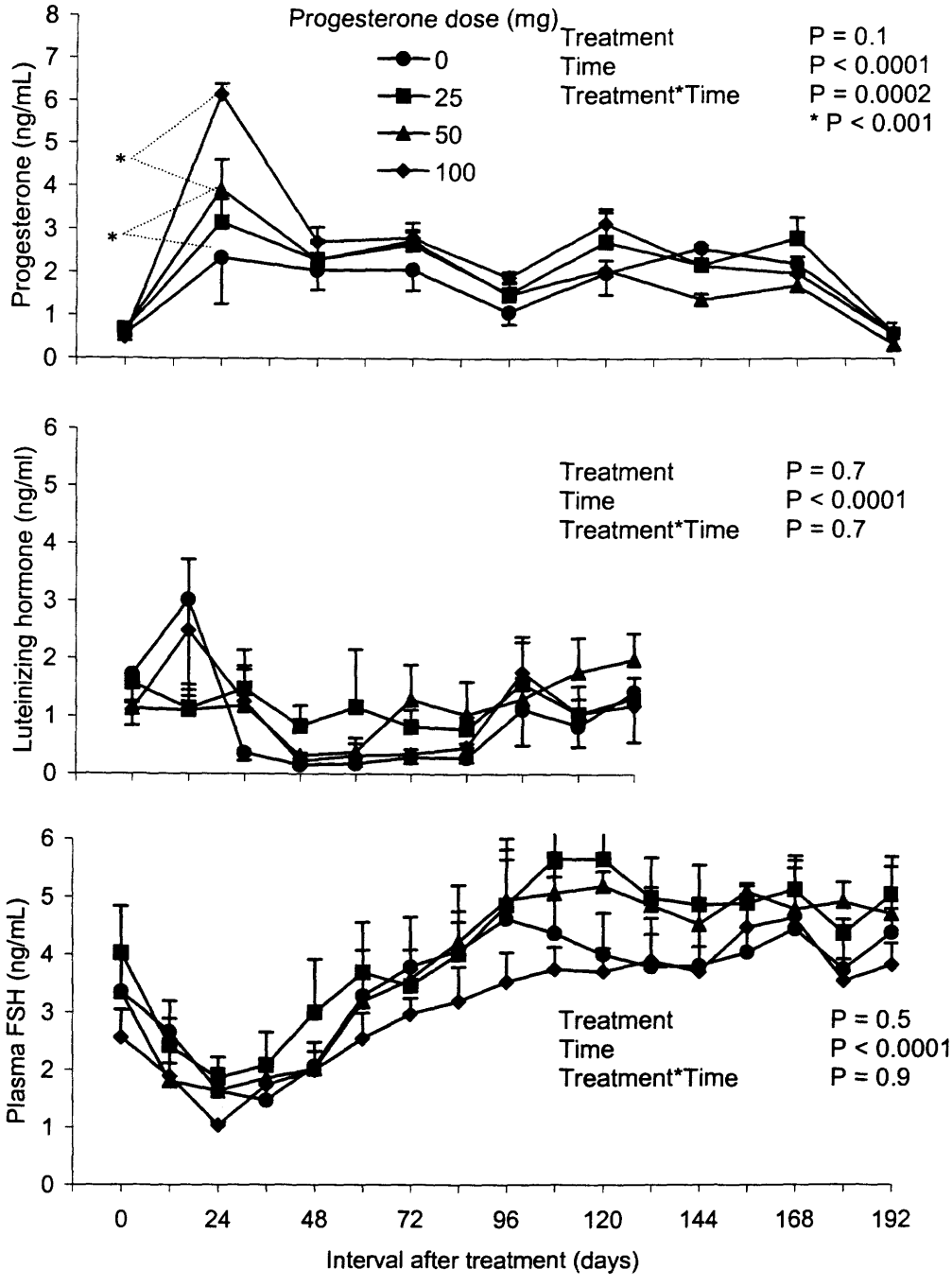


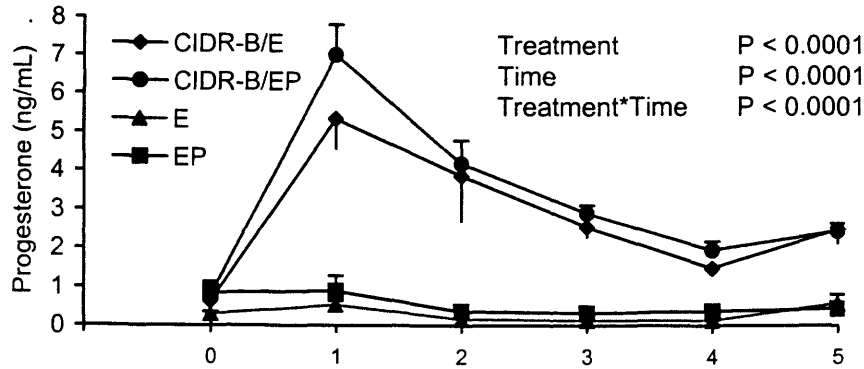
Figure 5.5 Mean (±SEM) plasma progesterone, LH and FSH concentrations in ovariectomized beef cows receiving a once-used CIDR-B device on Day 0 (beginning of the experiment) and concurrently given an i.m. injection of 5 mg E-17β plus 0, 25, 50, or 100 mg progesterone. The CIDR-B devices were removed on Day 7.

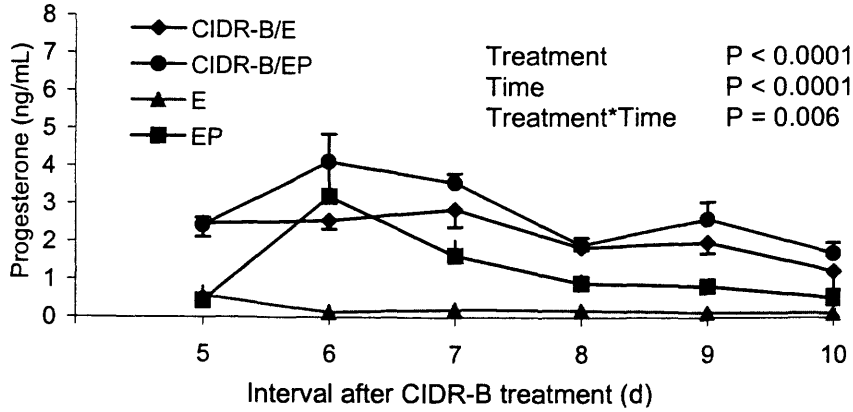
5.4.2 Experiment 2

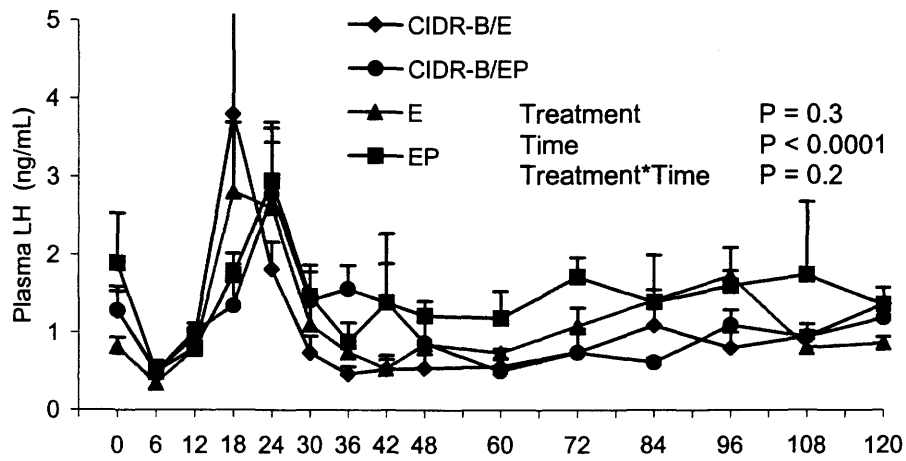
Progesterone. There was an effect of treatment ($P < 0.0001$), time ($P < 0.0001$), and treatment-by-time interaction ($P < 0.0001$) on plasma progesterone concentrations. Mean plasma progesterone concentrations from Day 0 to 5 were higher ($P < 0.0001$) in groups receiving CIDR-B devices than those that did not. On Day 6, progesterone concentrations in the two CIDR-B-treated groups and E-17β/P-treated groups were similar and higher ($P < 0.0001$) than those in the E-17β-treated group. Administration of 100 mg of progesterone increased ($P < 0.0001$) plasma concentration for approximately 2.0 ng/mL progesterone 24 h after treatment compared to the CIDR-B-treated group that did not receive on injection of progesterone. Progesterone concentrations in the E-17β/P group decreased ($P < 0.01$) to pretreatment concentrations by 72 h after treatment (Figure 5.6).

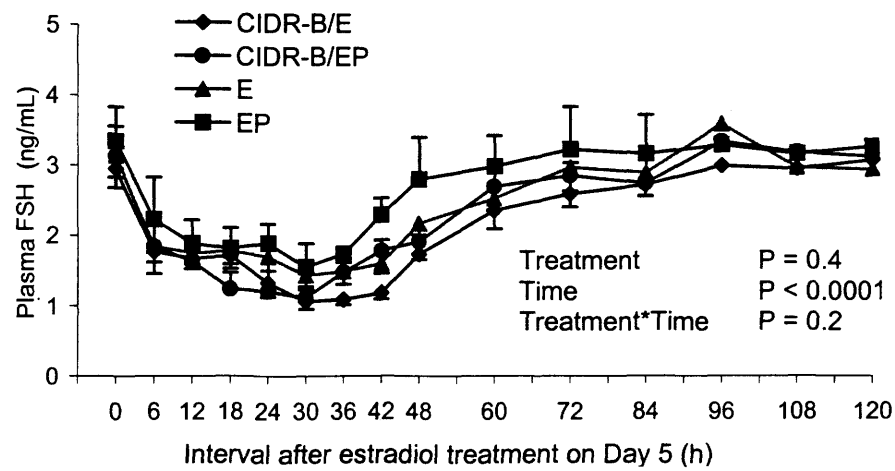
Luteinizing hormone. There was an effect of time ($P < 0.0001$), but no effect of treatment ($P = 0.3$), or treatment-by-time interaction ($P = 0.2$) on plasma LH concentrations. There was a decrease ($P < 0.05$) in plasma LH concentrations 6 h after treatment, followed by an increase ($P < 0.001$) at 18 and 24 h, and a decrease to baseline 36 h after treatment (Figure 5.6). There was a tendency ($P < 0.08$) for an effect of progesterone administration on the time to the median LH peak, which was 18 h in the groups treated with E-17β alone and 24 h for groups that also received progesterone along with E-17β (Figure 5.6).

Follicle stimulating hormone. There was an effect of time ($P < 0.0001$), but no effect of treatment ($P = 0.4$), or treatment-by-time interaction ($P = 0.8$) on plasma FSH concentrations. Mean (± SEM) plasma FSH concentrations in all groups decreased ($P < 0.0001$) 6 h after treatment with E-17β, remained low until 36 h, and then increased ($P < 0.005$) by 48 h after treatment (Figure 5.6).


Progesterone (ng/mL)
CIDR-B/E
CIDR-B/EP
E
EP
Treatment P < 0.0001
Time P < 0.0001
Treatment*Time P < 0.0001
0 1 2 3 4 5


Progesterone (ng/mL)
CIDR-B/E
CIDR-B/EP
E
EP
Treatment P < 0.0001
Time P < 0.0001
Treatment*Time P = 0.006
5 6 7 8 9 10
Interval after CIDR-B treatment (d)


Plasma LH (ng/mL)
CIDR-B/E
CIDR-B/EP
E
EP
Treatment P = 0.3
Time P < 0.0001
Treatment*Time P = 0.2
0 6 12 18 24 30 36 42 48 60 72 84 96 108 120


Plasma FSH (ng/mL)
CIDR-B/E
CIDR-B/EP
E
EP
Treatment P = 0.4
Time P < 0.0001
Treatment*Time P = 0.2
0 6 12 18 24 30 36 42 48 60 72 84 96 108 120
Interval after estradiol treatment on Day 5 (h)

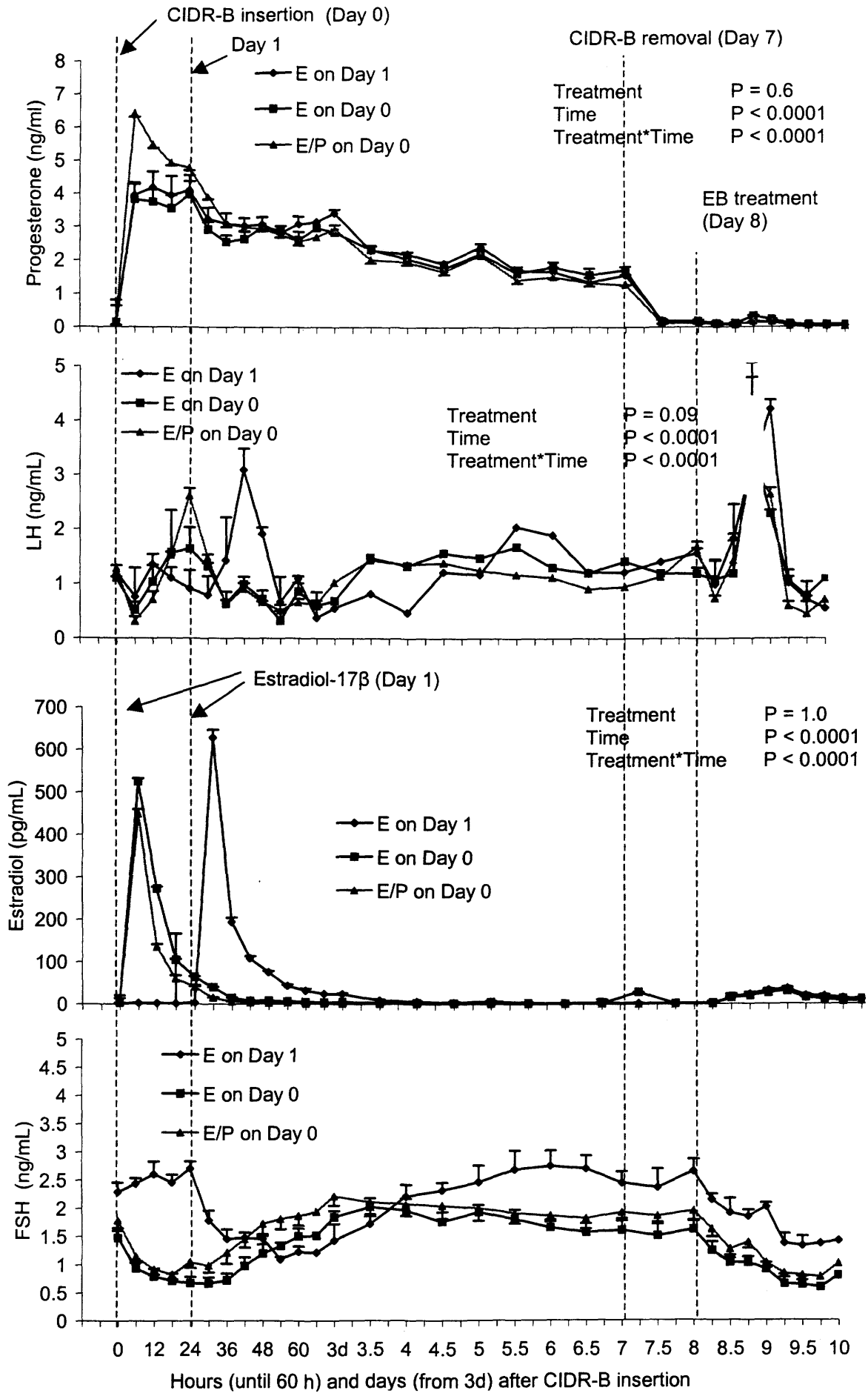
5.4.3 Experiment 3

Progesterone. There was no effect of treatment ($P = 0.6$), but there was an effect of time ($P < 0.0001$) and treatment-by-time interaction ($P < 0.0001$) on mean plasma progesterone concentrations. Plasma progesterone concentrations increased ($P < 0.0001$) by 6 h and were higher ($P < 0.0001$) in the E-17β/P group at 6 h after CIDR-B insertion than in the other groups. In all groups, plasma progesterone concentrations dropped by 84 h after treatment ($P < 0.0001$). After CIDR-B removal on Day 7, progesterone concentrations declined ($P < 0.0001$) to undetectable values by 12 h (Figure 5.7).

Luteinizing hormone. There was no effect of treatment ($P = 0.7$), but there was an effect of time ($P < 0.0001$) and a treatment-by-time interaction ($P = 0.05$) on mean plasma LH concentrations. When data for the first 48 h after treatment were normalized to E-17β treatment and analyzed, outcomes were very similar to those of the previous analysis (effects of treatment, $P = 0.80$; time, $P < 0.0001$; and treatment-by-time interaction, $P < 0.007$). Mean (± SEM) LH concentrations decreased ($P < 0.05$) 6 h after CIDR-B insertion in all treatment groups. Plasma LH concentrations then increased ($P < 0.05$) in all groups, peaking 18 h after estradiol injection in the E1 group (3.1 ± 0.4 ng/mL) and at 24 h in the EP (2.6 ± 0.4 ng/mL) and E0 (1.6 ± 0.4 ng/mL) groups (Figure 5.7). Then, plasma LH concentrations declined to levels lower than 1 ng/mL and remained around that concentration thereafter.

Estradiol. There was a tendency for an effect of treatment ($P = 0.09$), and there was an effect of time ($P < 0.0001$) and a treatment-by-time interaction ($P < 0.0001$) on mean plasma estradiol concentrations. In all groups, estradiol concentrations peaked ($P < 0.0001$) at 6 h after treatment with 5 mg E-17β, decreasing to baseline by 30 h ($P = 0.2$), 24 h ($P = 0.2$) and 30 h ($P = 0.2$) in E1, EP and E0 groups, respectively (Figure 5.7).

Follicle stimulating hormone. There was an effect of treatment ($P < 0.008$), time ($P < 0.0001$) and a treatment-by-time interaction ($P < 0.0001$) on mean plasma FSH concentrations (Figure 5.7, Table 5.1). When data for the first 48 h after estradiol treatment were normalized to time of E-17β treatment and analyzed, outcomes were very similar to those of the previous analysis (effects of treatment, $P = 0.007$; time, $P < 0.0001$; and treatment-by-time interaction, $P < 0.0001$, on plasma FSH concentrations). Mean (± SEM) plasma FSH concentrations were reduced ($P < 0.0001$) from those at the time of treatment (2.0 ± 0.1 ng/mL) by 6 h in all groups (1.3 ± 0.1 ng/mL; Table 5.1). By 30 h after treatment, plasma FSH values reached a nadir (average 0.9 ± 0.1 ng/mL; Figure 5.7). Plasma FSH concentrations began to increase after 30 h (Figure 5.7; Table 5.1).


CIDR-B insertion (Day 0)
Day 1
CIDR-B removal (Day 7)
E on Day 1
E on Day 0
E/P on Day 0
Treatment P = 0.6
Time P < 0.0001
Treatment*Time P < 0.0001
EB treatment (Day 8)
Progesterone (ng/ml)
LH (ng/mL)
Treatment P = 0.09
Time P < 0.0001
Treatment*Time P < 0.0001
Estradiol-17β (Day 1)
Estradiol (pg/mL)
Treatment P = 1.0
Time P < 0.0001
Treatment*Time P < 0.0001
FSH (ng/mL)
0 12 24 36 48 60 3d 3.5 4 4.5 5 5.5 6 6.5 7 7.5 8 8.5 9 9.5 10
Hours (until 60 h) and days (from 3d) after CIDR-B insertion

Hormone changes after EB treatment 24 h after CIDR-B removal. Mean plasma progesterone concentrations were less than 1.0 ng/mL 12 h after CIDR-B removal (Figure 5.8). Plasma estradiol concentrations increased ($P < 0.0001$) from 1.0 ± 0.4 pg/mL at the time of EB treatment (24 h after CIDR-B removal) to 15.0 ± 2.1 pg/mL by 6 h, continued to increase until 24 h (32.6 ± 2.4 pg/mL), and then decreased ($P < 0.01$). By 48 h after EB treatment, estradiol concentrations (9.9 ± 1.6 pg/mL) were still higher ($P < 0.05$) than those at the time of treatment. Plasma LH concentrations decreased ($P < 0.02$) for 6 h, and then increased at 18 h after EB treatment, declining to basal concentrations by 30 h after treatment. Plasma FSH concentrations decreased by 6 h ($P < 0.01$), reached a nadir by 30 h and then increased ($P < 0.04$) at 48 h after treatment.

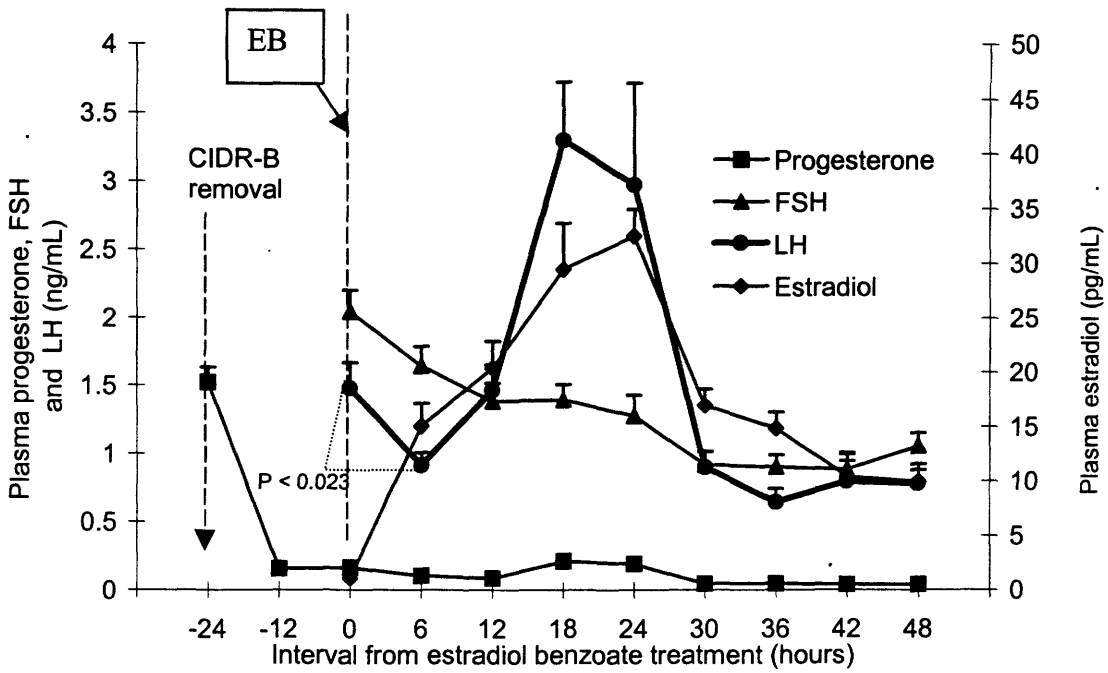


Figure 5.8 Mean (±SEM) plasma progesterone, estradiol, FSH and LH concentrations after treatment with 1 mg EB (EB) 24 h after CIDR-B removal in ovariectomized beef cows (n = 16).

Table 5.1 Mean (± SEM) FSH concentrations in ovariectomized beef cows (n = 16) treated with a CIDR-B device on Day 0 plus administration of E-17β on Day 0 or on Day 1 or E-17β and progesterone on Day 0. The CIDR-B devices were removed 7 d later.

End points	E1	E0	EP
FSH concentration at CIDR-B insertion	2.3 ± 0.1	1.5 ± 0.1	1.8 ± 0.1
Concentration at 6 h	1.8 ± 0.1	0.9 ± 0.1	1.2 ± 0.1
Nadir concentration (ng/mL)	1.1 ± 0.1[a]	0.7 ± 0.1[b]	0.8 ± 0.1[b]
Concentration at 36 h (ng/mL)	1.4 ± 0.1	0.9 ± 0.1	1.2 ± 0.1
Maximal concentration (ng/mL)	2.2 ± 0.1	2.0 ± 0.1	2.2 ± 0.1
Intervals (h) from E-17β			
to nadir concentration	30	30	18
to initial increase	48[a]	42[ab]	36[b]
to maximal concentration	72	84	72

[ab] Means with different superscripts differ significantly ($P < 0.05$).

5.4.4 Experiment 4

Progesterone. There was no effect of treatment ($P = 0.4$) or treatment-by-time interaction ($P = 0.9$), but there was an effect of time ($P < 0.0001$) on plasma progesterone concentrations (Figure 5.9). Progesterone concentrations increased from 0.4 ± 0.2 to 6.1 ± 0.2 ng/mL by 24 h after insertion of a CIDR-B device, decreasing to 4.9 ± 0.2 ng/mL at 72 h.

Luteinizing hormone. There was no effect of treatment ($P > 0.1$), but there was an effect of time ($P < 0.0001$) and treatment-by-time interaction ($P < 0.02$) on plasma LH concentrations (Figure 5.9). In the E-17β group, there was an increase ($P = 0.02$) in plasma LH concentrations by 24 h (from 0.8 ± 0.4 ng/mL to 2.1 ± 0.4 ng/mL), which was followed by a decrease ($P = 0.003$), reaching a nadir at 72 h after treatment. Mean plasma LH concentrations increased ($P = 0.05$) by 96 h after treatment. An increase ($P < 0.001$) in plasma LH concentrations was observed 12 h after CIDR-B removal in EB- and EV-treated groups.

Estradiol. There was no effect of treatment ($P = 0.6$), but there was an effect of time ($P < 0.0001$) and treatment-by-time interaction ($P < 0.0001$) on mean plasma estradiol concentrations (Figure 5.9). Plasma estradiol concentrations increased ($P < 0.0001$) from 0.4 ± 9.7 pg/mL at the time of CIDR-B insertion to 214.0 ± 9.7 pg/mL by 12 h in the E-17β group; 1.81 ± 9.7 pg/mL at treatment to 97.1 ± 9.7 pg/mL at 12 h in the EB group; and from 2.8 ± 8.9 pg/mL to 58.6 ± 8.9 pg/mL at 12 h in the EV group. Peak concentration of estradiol in cows treated with E-17β was the highest ($P < 0.0001$), while peak concentrations were higher in the EB group than in the EV group ($P < 0.005$). However, plasma estradiol concentrations decreased ($P < 0.0001$) at 24 h in the E17β group and by 72 h after treatment (5.2 ± 9.7 pg/mL) were lower ($P = 0.05$) than those in the EB (32.3 ± 9.7 pg/mL) or EV (28.9 ± 8.9 pg/mL) groups. Plasma estradiol concentrations were at baseline at 36 h in E-17β-treated animals and at 96 h in EB- and EV-treated cows ($P < 0.0001$) (Figure 5.9).

Follicle stimulating hormone. There was no effect of treatment ($P > 0.1$), but there was an effect of time ($P < 0.0001$) and treatment-by-time interaction ($P < 0.0001$) on plasma FSH concentrations. Plasma FSH concentrations decreased ($P < 0.0004$) by 12 h after estradiol treatment. Plasma FSH concentrations in the E-17β-treated group increased ($P < 0.0001$) by 60 h to pretreatment concentrations, and remained higher ($P < 0.05$) than in the EB and EV groups until the time of CIDR-B removal (Figure 5.9).

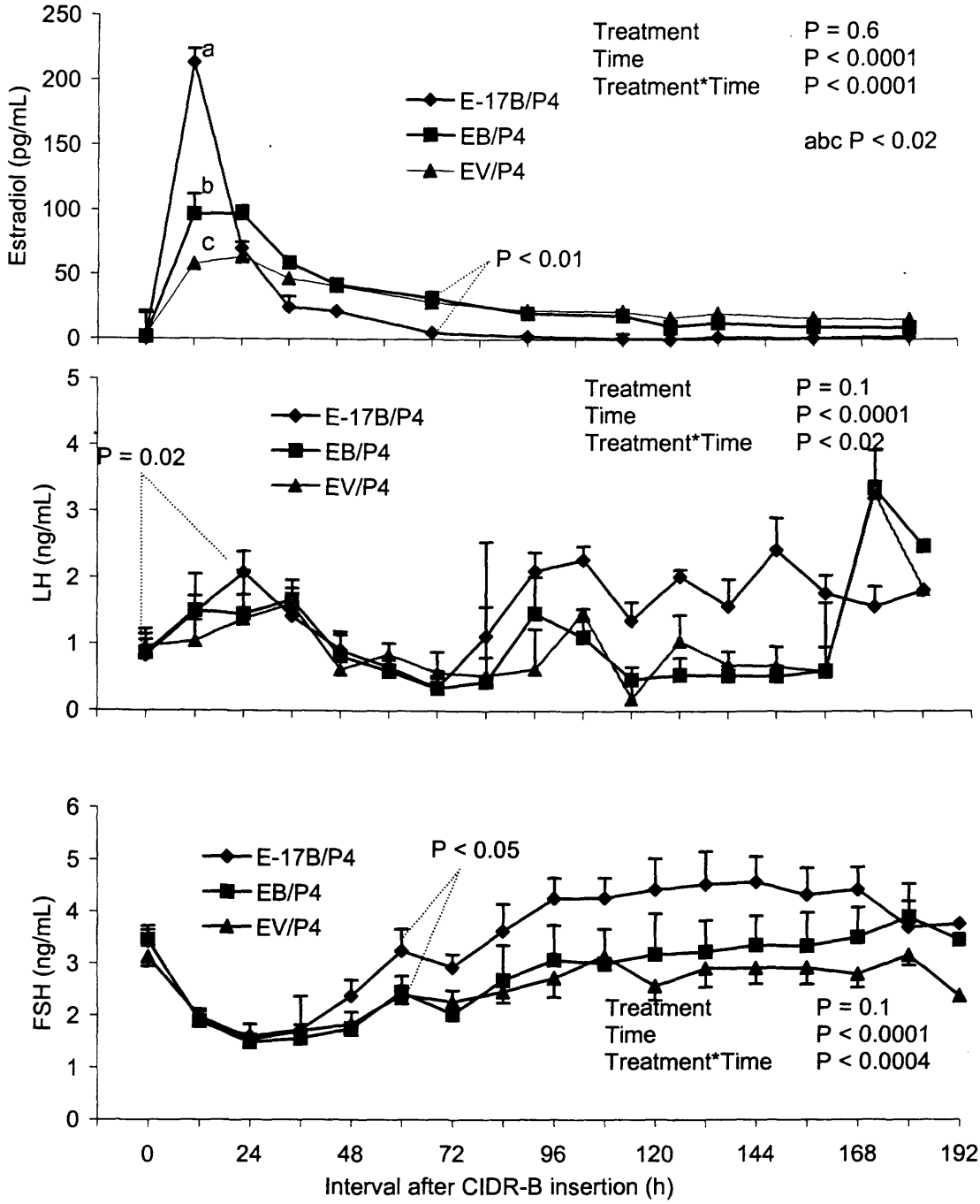


Figure 5.9 Mean (±SEM) plasma estradiol, LH and FSH concentrations in ovariectomized beef cows receiving a once-used CIDR-B device on Day 0 and 5 mg of E-17β, EB or EV plus 100 mg progesterone at that time. CIDR-B devices were removed on Day 7.

5.5 Discussion

The use of new CIDR-B devices resulted in plasma progesterone concentrations (~7 ng/mL) in ovariectomized cows, similar to those occurring between Days 11 and 15 of the estrous cycle in intact heifers (Kesner et al., 1982). In previous studies, plasma progesterone reached approximately 7 ng/mL 24 h after CIDR-B insertion in ovariectomized cattle (Macmillan et al., 1991; Macmillan and Peterson, 1993). High progesterone concentrations were detected as early as 6 h and remained elevated for at least 48 h after CIDR-B insertion, similar to previous reports in ovariectomized (Burke et al., 1996) and intact (Nation et al., 2000; Burke et al., 1999) cattle.

In our studies, 100 mg i.m. progesterone also increased progesterone concentrations in ovariectomized cows. Progesterone given i.m. to ovariectomized cows concurrently with a used CIDR-B device resulted in plasma progesterone concentrations equivalent to those in ovariectomized cows treated only with a new CIDR-B device; however, plasma progesterone remained elevated for a shorter interval (24 h after treatment). Daily progesterone injections given at the end of the estrous cycle increased the length of the estrous cycle in intact heifers (Christian and Casida, 1948) by suppressing estrus and ovulation. Furthermore, daily administration of high (300 mg/d) or 'physiological' (150 mg/d) doses of progesterone starting 6 days after ovulation resulted in regression of the dominant follicle of the first follicular wave (Adams et al., 1992b). More recently, it was reported that a single injection of progesterone induced regression of a persistent dominant follicle in melengestrol acetate-treated (200 mg progesterone; Anderson and Day, 1998; McDowell et al., 1998) or norgestomet-treated (100 mg progesterone; Cavalieri et al., 1998) heifers. Treatment with a CIDR-B device

for 24 h, or injections of progesterone in canola oil or alcohol/saline vehicle given on the Day 10 of a 17-day treatment with norgestomet implants in *Bos indicus* heifers resulted in the emergence of a new follicular wave, on average, 2.6 days after treatment (Cavalieri et al., 1998). Based on our studies in ovariectomized cows, the effect of progesterone on plasma LH concentrations became apparent after the effects of estradiol disappeared at 36 h, and then circulating LH remained low until 72 h after CIDR-B insertion. Therefore, the use of CIDR-B devices in intact animals would reduce the LH concentrations for 72 h and induce the regression of the LH-dependent dominant follicle.

The suppressive effect of progesterone on dominant or persistent follicles has been attributed to a decrease in concentrations and pulsatility of circulating LH resulting from treatment with exogenous progestins in the absence of a CL (Sirois and Fortune, 1990; Yelich et al., 1997). After selection, a dominant follicle has acquired LH receptors, becoming LH-dependent (Xu et al., 1995). A sudden rise in plasma progesterone concentrations may result in decreased plasma LH concentrations, changing the pattern of LH secretion and affecting the growth of the LH-dependent dominant follicle. In Experiments 2 and 3, the injection of progesterone and E-17β given concurrently with the insertion of a new or used CIDR-B device induced a decrease in mean LH concentrations at 6 h, followed by a surge-like increase at 18 to 24 h. In Experiment 3, although progesterone was injected at CIDR-B insertion in the EP group and a CIDR-B device was inserted one day before E-17β in the E1 group, plasma progesterone concentrations were not sufficient to prevent an E-17β-induced LH surge. However, it has been reported that a norgestomet implant placed one day before E-17β

treatment blocked the E-17β-induced LH surge in intact (Bó et al., 1994) or ovariectomized (Bolt et al., 1990) heifers. Those studies suggest that norgestomet released from the implant more effectively suppressed LH release than CIDR-B devices used in our studies in ovariectomized cows. However, the effect of body size or category of cattle used cannot be excluded as influencing the suppressive effect of progesterone. The dose of 100 mg progesterone used in our studies may be also marginal in suppressing LH release. The administration of 200 mg progesterone has been reported to delay the onset of estrus and ovulation of a newly recruited dominant follicle (Murray et al., 1998). Nevertheless, treatment with progesterone by injection should be considered when using estradiol to synchronize follicular wave emergence in stages of the estrous cycle in which endogenous progesterone is declining or may be low, e.g., end of the estrous cycle.

In studies in ovariectomized cattle, mean plasma LH concentrations decreased 2 to 8 h after CIDR-B insertion (Burke et al., 1996). In another study, serum LH concentrations declined slightly 3 h after E-17β administration and then increased in a surge-like fashion (Shoenemann et al, 1985). This phenomenon was also found in post-partum anestrous cows in which a transient initial decrease in mean LH concentrations and reduced LH pulsatility were observed after progesterone treatment (Nation et al., 2000). Both endpoints have been reported to return to pretreatment concentrations by Day 3 after CIDR-B insertion in ovariectomized cattle (Burke et al., 1996) and by Day 4 in intact cattle (Nation et al., 2000). Based on the results of our studies, it may be speculated that 5 mg E-17β at CIDR-B insertion masked the suppressive effect of progesterone on LH until at least 36 h after treatment. The combined effects of both

steroid hormones in suppressing both FSH and LH would cause regression of FSH- and LH-dependent follicles. This would be followed by a surge in FSH and emergence of a new follicular wave.

Plasma estradiol concentrations consistently peaked 6 h after E-17β treatment in ovariectomized cows and decreased gradually to baseline by 36 h. In previous studies, plasma estradiol concentrations increased dramatically by 2 (Bó et al., 2000a) and 6 h (Bó et al., 1994) after treatment with E-17β. In the present study, plasma estradiol concentrations increased more rapidly and reached a higher peak after E-17β treatment than after treatment with EB or EV. In addition, plasma estradiol concentrations returned to baseline 96 h after treatment with EB or EV, consistent with previous studies in ovariectomized (O'Rourke et al., 2000) and intact heifers (Bó et al., 1993). In addition, when 10 mg EB was given to ovariectomized heifers, plasma estradiol concentrations peaked at inconsistent times, returning to basal concentrations by 120 h in ovariectomized heifers (O'Rourke et al., 2000) or 160 h in intact cattle (Vynckier et al., 1990).

Peak concentrations of estradiol depended on the formulation used. Therefore, each estradiol form is expected to have different effects on gonadotrophin concentrations and ovarian follicles. As mentioned above, treatment with E-17β resulted in a sharp LH peak, while treatment with EB or EV did not. It is also noteworthy that in E-17β-treated cows, plasma LH concentrations increased to pretreatment concentrations 84 h after treatment, remaining higher than after treatment with the other estradiol esters, whereas circulating LH increased only after CIDR-B removal in the EB and EV

groups. This also occurred after treatment with the crystalline form of EB given intravaginally (Burke et al., 1996).

Increased concentrations of circulating estradiol suppressed plasma FSH concentrations. In ovariectomized cows with or without CIDR-B devices, 5 mg E-17β reduced plasma FSH concentrations from 6 to 48 h after treatment. Similar results have been observed after treatment 10 mg E-17β (with or without a norgestomet implant) in ovariectomized heifers in which plasma FSH concentrations did not reach pre-treatment concentrations by 60 h (Bolt et al., 1990). If extrapolated to intact animals, suppression of plasma FSH concentrations for this interval may result in the emergence of a follicular wave one day later (i.e., 3- to 4-day interval from treatment to wave emergence; Bó et al., 1995a). In Experiment 4, plasma FSH concentrations increased earlier and reached higher peak concentrations in the E-17β group than in the EB or EV groups; FSH returned to pre-treatment concentrations by 2.5 d (60 h) after treatment in the E-17β group while in the EB and EV groups, plasma FSH began to increase by 96 h after treatment. A similar result was shown after 5 mg EV was given to cyclic Holstein cows (Barnes et al., 1981). The delayed surge in plasma FSH concentrations may explain delayed wave emergence observed in intact cattle after treatment with 5 mg of EB (Bó et al., 1996) or EV (Bó et al., 1993; Mapletoft et al., 1999). In addition, there may be increasing variability in individual responses due to different estradiol concentrations. However, after administration of 1 mg EB, plasma FSH concentrations had a similar pattern of decline to that after 5 mg E17β (Experiment 3). Similar FSH suppression and plasma FSH profiles following an injection of 1 mg EB were observed in ovariectomized heifers (O'Rourke et al., 2000). Results indicate that FSH secretion

and release are estradiol dose-dependent and that different estradiol esters suppress FSH release differently, depending on the length of time that estradiol remains elevated in the circulation. Therefore, a small dose of EV may have a similar effect on gonadotrophin release as a small dose of EB.

In summary, CIDR-B devices were effective in inducing increased circulating concentrations of progesterone similar to those found during the luteal phase of the estrous cycle. However, plasma progesterone concentrations remained elevated for 48 h, and were not capable of blocking estradiol-induced LH release, even though supplementary progesterone was injected concurrently with estradiol. Estradiol-17β and progesterone resulted in LH and FSH suppression by 6 h after treatment. However, suppressive effects were followed by LH release in a surge-like manner while FSH concentrations remained depressed until 36 h after treatment. Therefore, treatment with estradiol and CIDR-B devices in intact cattle may result in suppression of small follicles (by estradiol) and large dominant follicles (by progesterone), which would be followed by the emergence of a new follicular wave following the resurgence of FSH. Additionally, EB and EV had a longer suppressive effect than E-17β on circulating FSH concentrations. However, at a lower dose, EB suppressed plasma FSH concentrations for a shorter interval than a high dose and after CIDR-B removal, resulted in an LH surge that could induce ovulation in intact cattle.

6.0 EFFECTS OF ESTRADIOL-17β AND ESTRADIOL BENZOATE ON OVARIAN FOLLICULAR DYNAMICS IN CIDR-B-TREATED BEEF CATTLE.

6.1 Abstract

The objectives were to: 1) compare the effects of either estradiol-17β (E-17β) or estradiol benzoate (EB) in the synchronization of ovarian follicular wave emergence and ovulation in CIDR-B-treated beef cattle; and 2) determine the timing of LH release and ovulation of the dominant follicle of a synchronized follicular wave after administration of EB at various intervals after progesterone withdrawal. In Experiment 1, non-lactating Hereford cows (n = 29) received a new CIDR-B intravaginal insert on Day 0 (beginning of the experiment), and were randomly assigned to receive i.m. injections of 1 or 5 mg of E-17β or EB on Day 1. On Day 8, CIDR-B devices were removed and PGF was given i.m. Transrectal ultrasound examinations were done once daily from 2 days before CIDR-B insertion to 2 days after CIDR-B removal and then twice daily to ovulation. There was no difference among groups in the interval from estradiol treatment to follicular wave emergence (P = 0.5). Treatment with 5 mg of E-17β resulted in the least variable interval to wave emergence (P < 0.005), compared with the other treatment groups which were not different (P = 0.1). For the interval from CIDR-B removal to ovulation, there were no differences among groups for either means or variances (P = 0.5 and P = 0.1, respectively). In Experiment 2, beef heifers (n = 32) received a once-

used CIDR-B device on Day 0 (beginning of the experiment) and were randomly assigned to receive i.m. injections of either 5 mg E-17β plus 100 mg progesterone or 1 mg EB plus 100 mg progesterone i.m. On Day 7, CIDR-B devices were removed and all heifers received PGF. On Day 8 (24 h after CIDR-B removal), each group was randomly subdivided to receive either 1 mg E-17β or 1 mg EB i.m. Treatment with either form of estradiol did not affect the mean (± SEM) interval from treatment to follicular wave emergence (4.1 ± 0.2 days, P = 0.7) or overall median interval from CIDR-B removal to ovulation (76.6 h; P = 0.7) or the range (72 to 120 h; P = 0.08). In Experiment 3, follicular wave emergence was induced in heifers (n = 40) with a once-used CIDR-B device and 5 mg E-17β plus 100 mg progesterone i.m. on Day 0. On Day 7, CIDR-B devices were removed, PGF was administered and heifers were assigned to four groups. The Control group received no treatment and the other three groups were given 1 mg i.m. EB at 12, 24, or 36 h after CIDR-B removal. Ultrasonographic examinations were done once daily from Day 0 to the day of EB treatment (to monitor ovarian changes) and then twice daily from EB treatment to ovulation. Blood samples were taken every 6 h from EB treatment to ovulation. Plasma estradiol concentrations increased within 6 h (P < 0.001) after EB treatment in all three groups. Plasma LH concentrations peaked 78.0 ± 23.0 h after CIDR-B removal in the Control group compared to 37.8 ± 8.5, 44.4 ± 10.3, and 51.0 ± 5.1 h after CIDR-B removal in groups treated with EB at 12, 24, or 36 h, respectively (means, P < 0.001; variances, P < 0.001). Mean (± SEM) intervals from CIDR-B removal to ovulation were 102.0 ± 6.7, 63.6 ± 3.6, 81.6 ± 3.5, and 78.0 ± 4.1 h for Control heifers or those treated with EB at 12, 24, and 36 h, respectively (P < 0.05). In summary, both E-17β and EB effectively synchronized ovarian follicular wave

emergence and ovulation in CIDR-B-treated cattle. The interval from CIDR-B removal to ovulation was significantly shorter and less variable in all EB-treated groups compared to the Control group and ovulation was hastened in heifers that received EB at 12 h compared to 24 or 36 h after CIDR-B removal.

6.2 Introduction

There is widespread use of CIDR-B devices for controlling estrus and ovulation in cattle. Estradiol has been used to synchronize follicular wave emergence (Bó et al., 1991, 1993). Numerous studies have investigated the use of different estradiol preparations in progestin-based synchronization programs (Lammoglia et al., 1998; Burke et al., 1999; Martínez et al. 2000). The administration of 5 mg estradiol-17β (E-17β) to progestin-treated heifers (Bó et al., 1994) suppressed small antral follicles, followed by the emergence of a new follicular wave, on average, 4.3 days later (Bó et al., 1995a), whereas the same dose of estradiol benzoate (EB) in Hereford cows induced emergence of a new follicular wave 5.4 days later (Bó et al., 1996). More recently, treatment with 1, 2.5 or 5 mg EB (and 50 mg of progesterone) resulted in a median day of follicular wave emergence of 4.0 days in CIDR-B-treated beef cows; this interval was significantly more synchronous in cows given 2.5 mg than in those given 5 mg EB (Caccia and Bó, 1998).

Estradiol benzoate has been used to induce estrus in prostaglandin F2α- (PGF) treated cattle (Welch et al., 1975; Peters et al., 1977; Dailey et al., 1983). In CIDR-B-treated cattle, the administration of 0.38 (heifers) or 1.0 (cows) mg EB 24 to 30 h after

CIDR-B removal resulted in estrus in 86 and 100% of the cattle, respectively (Lammoglia et al., 1998). Furthermore, EB treatment induced a synchronous LH surge (between 16 and 20 h after treatment), resulting in significantly higher pregnancy rates than in non-treated cattle (Lammoglia et al., 1998). However, a critical comparison between E-17β and EB has not yet been done and the effect of the interval between CIDR-B removal and EB treatment on the timing and synchrony of ovulation has not been studied.

Three experiments were conducted to compare the effects of E-17β and EB on the synchronization of follicular wave emergence and ovulation in CIDR-B-treated beef heifers and to determine the timing of LH release and ovulation after the administration of EB at various intervals after progesterone withdrawal.

6.3 Materials and Methods

6.3.1 Experiment 1

Non-lactating Hereford cows (n = 29) 3 to 6 years of age, weighing 450 to 550 kg) and kept in pasture consisting of oats and native grass during the winter in Córdoba, Argentina (31° 24' S, 64° 11' W, 400 meters above sea level) were used in this experiment. Cows received a new progesterone-releasing vaginal device (CIDR-B; Vetrepharm Canada Inc, Belleville ON, Canada) intravaginally on Day 0 (beginning of the experiment), and were assigned to four groups to receive an i.m. injection of 1 or 5 mg of E-17β or EB (both from Sigma-Aldrich Canada Ltd, Oakville, ON, Canada) in 2

mL canola oil on Day 1 (2x2 factorial layout). On Day 8, CIDR-B devices were removed and 500 μg of cloprostenol (PGF; Estrumate, Schering Canada Inc, Pointe-Claire, PQ, Canada) was given i.m. Transrectal ultrasonographic examinations were done by a single operator using a real-time B-mode scanner with a 5.0 MHz linear-array transducer (Aloka SSD-500, ISM Inc, Edmonton, AB, Canada). Ultrasonography was performed once daily from 2 days before CIDR-B insertion to 2 days after CIDR-B removal, and then every 12 h to ovulation or to 7 days after device removal if ovulation did not occur. During each examination, a sketch of each ovary was made and the diameter and location of follicles 3 mm in diameter were recorded. Ovulation was defined as the abrupt disappearance (from one examination to the next) of a previously identified follicle greater 8 mm in diameter. The dominant follicle of a wave was defined as the follicle that reached the largest diameter. The day of wave emergence was retrospectively defined as the day when the dominant follicle was first detected at a diameter of 4 to 5 mm. However, if the dominant follicle was not detected until it reached 6 or 7 mm, the previous day was considered the day of wave emergence (Ginther et al., 1989b). Two-way analysis of variance was used to compare the effects of dose (1 or 5 mg), estradiol formulation (E-17β or EB) and the dose-by-formulation interaction. The LSD multiple comparison test was used to locate differences among groups. Statistical analyses were conducted with a commercial program for statistical analysis (Statistix Student Version, version 2.0, Analytical Software, Tallahassee, Florida, USA).

6.3.2 Experiment 2

Thirty-two pubertal beef heifers were housed outdoors in feedlot pens at the Goodale Research Farm, University of Saskatchewan and fed barley silage, with free access to water. Heifers had a once-used CIDR-B inserted intravaginally on Day 0 (beginning of the experiment) and were assigned to four groups; half of the heifers received an i.m. injection of 5 mg E-17β plus 100 mg of progesterone in 2 mL canola oil and the other half received 1 mg EB plus 100 mg of progesterone in 2 mL canola oil. After CIDR-B removal and PGF treatment (Day 7), the heifers received i.m. injections of either 1 mg E-17β or EB in 2 mL canola oil on Day 8, (24 h after CIDR-B removal). Transrectal ultrasonography was done once daily from Day 0 to Day 9 to monitor ovarian follicular development, and every 12 h thereafter to detect ovulation. Data prior to the second injection of estradiol were dependent only on the initial treatment, therefore, diameter of the dominant follicle and CL at the time of the first estradiol treatment and the interval from estradiol treatment to the emergence of the next follicular wave were compared as two groups (i.e., E-17β or EB). Student's *t*-tests were used to compare, between groups, the diameters of the dominant follicle and the CL at the time of treatment with estradiol or PGF, the interval from estradiol treatment to the emergence of the next follicular wave, and the interval from CIDR-B removal to ovulation. Bartlett's test of homogeneity of variance was used to compare the variability of the day of wave emergence. Statistical analyses were conducted with a commercial program for statistical analysis (Statistix Student Version).

6.3.3 Experiment 3

Beef heifers (n = 40), housed outdoors in feedlot pens at the Goodale Research Farm, University of Saskatchewan and fed barley silage, received a once-used CIDR-B device and an i.m. injection of 5 mg of E-17β plus 100 mg of progesterone in 2 mL canola oil on Day 0 (beginning of the experiment). On Day 7, CIDR-B devices were removed, 500 μg PGF was administered concurrently. Heifers were randomly assigned to four groups to receive no treatment (Control), or an i.m. injection 1 mg of EB (in 2 mL canola oil) 12, 24, or 36 h after CIDR-B removal (Figure 5.1). Transrectal ultrasonographic examinations were done once daily from Day 0 to Day 7 to monitor ovarian follicular development and twice daily from EB treatment to ovulation using a real-time, B-mode scanner (Aloka SSD-500) with a 7.5 MHz linear-array transducer. In the Control group, examinations were performed as in the group given EB 24 h after CIDR-B removal. Blood samples were taken daily (for determination of estradiol and progesterone concentrations) and every 6 h for 48 h after EB administration (for LH concentrations).

Blood processing. Blood samples for estradiol, progesterone, LH and FSH radioimmunoassay were collected in heparinized tubes, kept at approximately at 4°C, centrifuged within 4 h after collection, and plasma frozen until assayed. Plasma LH concentrations were determined by double antibody radioimmunoassay (Honaramooz et al., 2000) and were expressed in terms of NIDDK-bLH4. The sensitivity of the LH assay, assessed as the lowest concentration of LH capable of displacing labeled LH from the antibody, was 0.06 ng/mL. Intra- and inter-assay coefficients of variation were 5.9 and 2.5%, respectively, for reference sera with mean LH concentrations of 0.35 and 0.95

ng/mL. Plasma concentrations of FSH were determined using a liquid-phase antibody radioimmunoassay (Rawlings et al., 1984; Honaramooz et al., 1999). Assays for progesterone and estradiol were conducted as described by Rawlings et al. (1984). Progesterone was extracted with 3 mL of hexane added to 200 μL aliquots of plasma. The sensitivity of the progesterone radioimmunoassay was 0.1 ng/mL. Intra- and inter-assay coefficients of variation were 12.0 and 13.8% (means, 1.36 and 0.44 ng/mL, respectively). Estradiol was extracted with ethyl ether. The sensitivity of the assay was 0.5 pg/mL and the intra- assay coefficient of variation was 6.3 and 4.0% (means 8.6 and 207.2 pg/mL, respectively).

Maximal mean concentrations of plasma estradiol or LH were calculated with data from all heifers at the time of the highest hormone concentration in the treatment group, whereas mean peak concentrations were calculated with the highest concentration of an individual heifer within a treatment group.

Hormonal data were analyzed using the GLM procedure (main effects: treatment, time and treatment-by-time interaction; SAS/STAT User's Guide, 1990). Main effects and mean LH concentrations during the LH surge were compared by the protected LSD test. One-way analysis of variance was used to compare the interval from EB treatment to ovulation and the LSD multiple range test was used to compare means. Variability in ovulation time (expressed as variance) was compared by Bartlett's test. Analyses of data points were conducted with a commercial program for statistical analysis (Statistix Student Version).

The protocols for these experiments were approved by University of Saskatchewan Animal Care Committee.

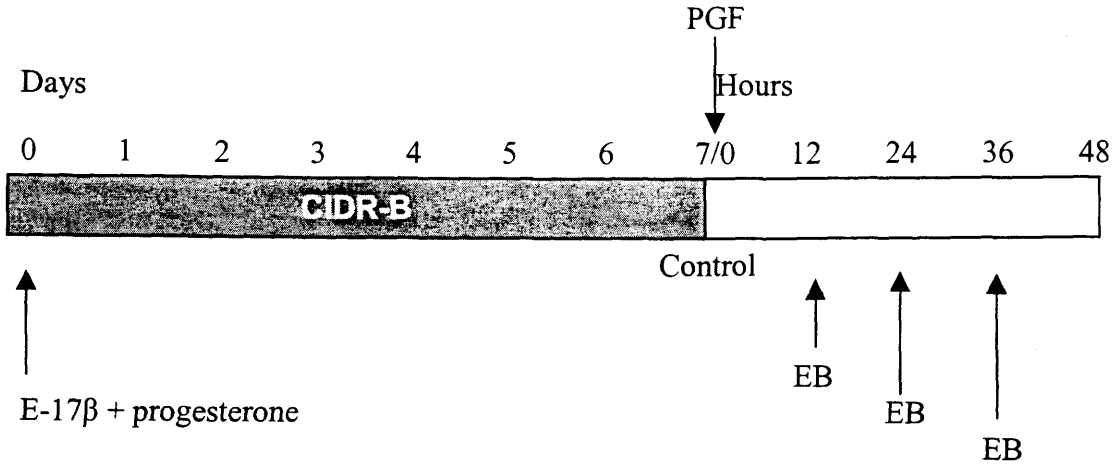


Figure 6.1 Treatment schedules of beef heifers treated with a once-used CIDR-B device on Day 0 along with the administration of E-17β and progesterone. The CIDR-B devices were removed on Day 7. An i.m. injection of 1 mg of EB was given 12, 24, or 36 h after CIDR-B removal. Blood samples were collected every 6 h from EB treatment to ovulation.

6.4 Results

6.4.1 Experiment 1

One cow treated with 1 mg E-17β and two cows treated with 5 mg EB had emergence of a follicular wave 2 days before treatment (Day -2); the future dominant follicle was not suppressed, and continued growing until it ovulated (after CIDR-B removal). Statistical analyses were done both for the complete data set, and for the data excluding these 3 cows and the results are shown in Table 6.1. The administration of 5 mg of E-17β resulted in the least variable interval to wave emergence ($P < 0.005$), compared with the other treatment groups which were not different ($P > 0.1$). There was

an effect of dose ($P < 0.05$) and estradiol formulation ($P < 0.01$) on the diameter of the dominant follicle at CIDR-B removal; cows treated with E-17β had a larger dominant follicle (10.4 ± 0.5 mm) than those treated with EB (7.9 ± 0.6 mm). However, there was no difference on the diameter of the dominant follicle at the time of CIDR-B removal between cows treated with 1 mg of EB and those treated with 1 or 5 mg E-17β ($P > 0.05$). The diameter of the CL at CIDR-B removal was the smallest in cows treated with 1 mg E-17β compared to the rest of treatment groups ($P < 0.03$). There was no difference among groups in the mean ($P = 0.5$) or variability ($P = 0.1$) of the interval from CIDR-B removal to ovulation (Table 6.1).

Table 6.1 Mean (± SEM) diameter of the dominant follicle (DF) and intervals from estradiol benzoate (EB) treatment to follicular wave emergence and from CIDR-B removal and PGF treatment to ovulation in non-lactating Hereford cows treated with a CIDR-B device one day before EB treatment (Experiment 1).

	5 mg E-17β	1 mg E-17β	5 mg EB	1 mg EB
No. of cows	8	7	6	8
Diameter (mm) of DF at				
Estradiol treatment	11.4 ± 1.1	10.8 ± 1.0	10.1 ± 1.3	9.8 ± 1.3
CIDR-B removal	9.4 ± 0.5^{a}	10.9 ± 0.9^{a}	7.0 ± 0.9^{b}	8.8 ± 0.7^{ab}
Diameter (mm) of the CL at				
Estradiol treatment	19.1 ± 1.5	20.4 ± 1.5	18.6 ± 2.6	17.0 ± 2.1
CIDR-B removal	15.1 ± 1.8^{a}	10.3 ± 1.1^{b}	14.1 ± 1.6^{ab}	15.1 ± 2.0^{ab}
Interval (d) from estradiol treatment to wave emergence				
Mean (± SEM)	3.6 ± 0.2^{x}	4.1 ± 0.3^{y}	5.5 ± 0.5^{y}	3.6 ± 0.5^{y}
Range	3 to 4	3 to 5	4 to 8	2 to 6
Interval (h) from CIDR-B removal to				
Ovulation	99.0 ± 7.4^{xy}	80.6 ± 4.3^{x}	98.0 ± 12.9^{y}	94.5 ± 8.3^{xy}
Range	72 to 132	72 to 96	72 to 144	72 to 144
No. cows ovulating	8	7	6	8

^{ab} Means with superscripts not in common are different ($P < 0.01$).
^{xy} Variances with superscripts not in common are different ($P < 0.05$).

6.4.2 Experiment 2

In one heifer that received EB and one that received E-17β, treatment was not followed by wave emergence. Therefore, data analyses were done for all heifers and for heifers excluding these two non-responders, and the results are shown in Table 5.2. Mean (± SEM) diameter of the dominant follicle and of the CL at CIDR-B insertion (12.0 ± 0.5 mm; 19.7 ± 0.7 mm, respectively) or removal (9.5 ± 0.3; 14.3 ± 0.9 mm, respectively) did not differ (P = 0.3, P = 0.4, respectively) between estradiol groups.

The mean (P = 0.7) and variability (P = 0.6) of the interval from treatment to follicular wave emergence were not different between the E-17β and EB groups. The interval from CIDR-B removal to ovulation also did not differ between groups (P = 0.7).

Table 6.2 Mean (± SEM) intervals (and their range) from estradiol treatment to follicular wave emergence and from CIDR-B removal to ovulation in CIDR-B-treated beef heifers after administration of estradiol-17β or estradiol benzoate to synchronize follicular wave emergence and ovulation (Experiment 2).

	Estradiol-17β	Estradiol benzoate
Induction of follicular wave emergence		
No. of heifers	16	16
Interval (d) from estradiol treatment to		
Wave emergence	3.4 ± 0.5	3.7 ± 0.6
Range	-3 to 5	-5 to 6
Excluding non-responding heifers		
No. of heifers	15	15
Wave emergence (d)	3.9 ± 0.2	4.3 ± 0.2
Range (d)	3 to 5	3 to 6
Induction of ovulation		
Interval (h) from CIDR-B removal to		
Ovulation	75.8 ± 3.0	77.3 ± 1.9
Range	72 to 120	72 to 96

6.4.3 Experiment 3

The overall mean (±SEM) interval from E-17β treatment to the emergence of the new follicular wave was 4.1 ± 0.2 d. The diameter of the dominant follicle at the time of EB treatment at 12, 24 and 36 h was 9.4 ± 0.7, 9.8 ± 0.2, and 10.7 ± 0.4 mm, respectively (P = 0.3). There was an effect of time of estradiol treatment on the mean and variance of the interval from CIDR-B removal to estrus (P = 0.001; P = 0.004, respectively) and to ovulation (P < 0.001; P < 0.07, respectively; Table 5.3).

Table 6.3· Mean (± SEM) interval (h) from CIDR-B removal to estrus and to ovulation in Control heifers and those treated with estradiol benzoate (EB) at 12, 24, or 36 h after CIDR-B removal (Experiment3).

		EB administered at		
	Control	12 h	24 h	36 h
No. of heifers	10	10	10	10
Interval (h) from CIDR-B removal to				
Estrus	63.6 ± 7.0^{ax}	33.3 ± 1.4^{by}	45.0 ± 3.3^{by}	52.0 ± 2.7^{aby}
Ovulation	102.0 ± 6.7^{cy}	63.6 ± 3.6^{ax}	81.6 ± 3.5^{bx}	78.0 ± 4.1^{bxy}
Median	96	60	72	84
Range	84 to 144	60 to 84	72 to 108	48 to 96

^{abc} Means and ^{xy} variances with superscripts not in common are different (in interval to estrus, P < 0.05 and P < 0.01, respectively; in interval to ovulation, P < 0.05; P < 0.07, respectively).

Progesterone. Mean (± SEM) plasma progesterone concentrations did not differ among groups (P = 0.9). Combined for all heifers, progesterone concentrations were 3.0 ± 0.3, 4.3 ± 0.3, and 0.7 ± 0.2 ng/mL, respectively at CIDR-B insertion, CIDR-B removal, and 24 h after CIDR-B removal (Table 5.4).

Estradiol. Plasma estradiol concentrations increased (P < 0.001) within 6 h after treatment with 1 mg EB, which differed from those in the Control group (P = 0.0002). Peak concentrations are shown in Table 5.4. Maximal mean estradiol concentrations were detected at 12 (35.9 ± 3.8 pg/mL), 18 (39.6 ± 3.8 pg/mL) and 12 h (42.8 ± 3.8 pg/mL) after treatment with EB at 12, 24, or 36 h, respectively, and all were higher (P < 0.0001) than in the Control group (peak = 5.0 ± 2.1 pg/mL) at 48 h after CIDR-B removal (Figure 5.2; Table 5.5).

Luteinizing hormone. Peak concentrations of plasma LH are shown in Table 5.4. Maximal mean (±SEM) plasma LH concentrations after CIDR-B removal did not differ (P < 0.14) between the Control group and EB-treated groups (2.8 ± 0.3 ng/mL). There was a time of treatment effect on the interval from EB to LH peak (P = 0.02); the interval was shorter (P = 0.007) in the group treated with EB at 36 h (15.0 ± 1.6 h) than in the group treated with EB at 12 h (25.8 ± 2.7 h), whereas the group treated at 24 h (20.4 ± 3.2 h) was not different from either. The interval from the estradiol peak to the LH surge in groups treated with EB at 12, 24, and 36 h was less variable (P = 0.02) than in the Control group (21.6 ± 7.4 h; Figure 5.2 and Table 5.5).

Table 6.4 Mean (± SEM) concentrations of progesterone, estradiol and LH in heifers treated with estradiol benzoate (EB) at 12, 24 or 36 h after CIDR-B removal in beef heifers.

End points	Control	Estradiol benzoate administered at		
		12 h	24 h	36h
No. of heifers	10	3	3	3
Plasma progesterone (ng/mL)				
CIDR-B insertion	3.6 ± 0.8	2.9 ± 0.9	2.7 ± 0.7	2.8 ± 0.9
CIDR-B removal	3.8 ± 0.8	4.7 ± 0.9	4.4 ± 0.9	4.5 ± 1.0
24 h after CIDR-B removal	0.7 ± 0.1	0.9 ± 0.3	0.6 ± 0.1	0.5 ± 0.1
Peak plasma estradiol (pg/mL)	6.9 ± 1.1[a]	37.7 ± 0.4[b]	45.3 ± 3.9[b]	43.7±8.9[b]
Peak plasma LH (pg/mL)	3.3 ± 0.4	4.2 ± 0.5	3.9 ± 0.2	3.8 ± 0.4

[ab] Means with different superscripts differ ($P < 0.05$).

Table 6.5 Mean (± SEM) intervals from CIDR-B removal and estradiol benzoate (EB) treatment to estradiol and LH peaks and to estrus and ovulation after treatment with EB at 12, 24 or 36 h after CIDR-B removal in beef heifers.

End points	Control	Estradiol benzoate administered at 12 h	24 h	36h
Interval (h) from CIDR-B removal to				
Estradiol peak	56.4 ± 3.6[a]	24.0 ± 0.0[b]	42.0 ± 3.5[c]	52.0 ± 4.0[ac]
No. of heifers	10	3	3	3
LH peak	78.0 ± 7.27[a]	37.8 ± 2.7[b]	44.4 ± 3.3 [bc]	51.0 ± 1.6[c]
No. of heifers	10	10	10	10
Estrus	63.6 ± 6.9[a]	33.3 ± 1.5[b]	45.0 ± 3.3[bc]	52.0 ± 2.8[ac]
No. of heifers	10	9	10	9
Ovulation	102.0 ± 6.8[a]	63.6 ± 3.6[b]	81.6 ± 3.5[c]	78.0 ± 4.1[c]
No. of heifers	10	10	10	10
Interval (h) from estradiol injection to				
Estradiol peak		12.0 ± 0.0	18.0 ± 3.5	16.0 ± 4.0
No. of heifers		3	3	3
LH peak		25.8 ± 2.7[a]	20.4 ± 3.3[ab]	15.0 ± 1.6[b]
No. of heifers		10	10	10
Estrus (h)		20.0 ± 2.2	21.0 ± 3.3	16.0 ± 2.8
No. of heifers		9	10	9
Ovulation		51.6 ± 3.6[ab]	57.6 ± 3.5[b]	42.0 ± 4.1[a]
No. of heifers		10	10	10
Interval (h) from LH peak to				
Ovulation	24.0 ± 5.4[d]	25.8 ± 5.0[d]	37.2 ± 2.0[e]	28.5 ± 3.9[de]
No. of heifers	10	10	10	10

[abc] Means with superscripts not in common differ ($P < 0.05$).
[de] Means with superscripts not in common differ ($P < 0.06$).

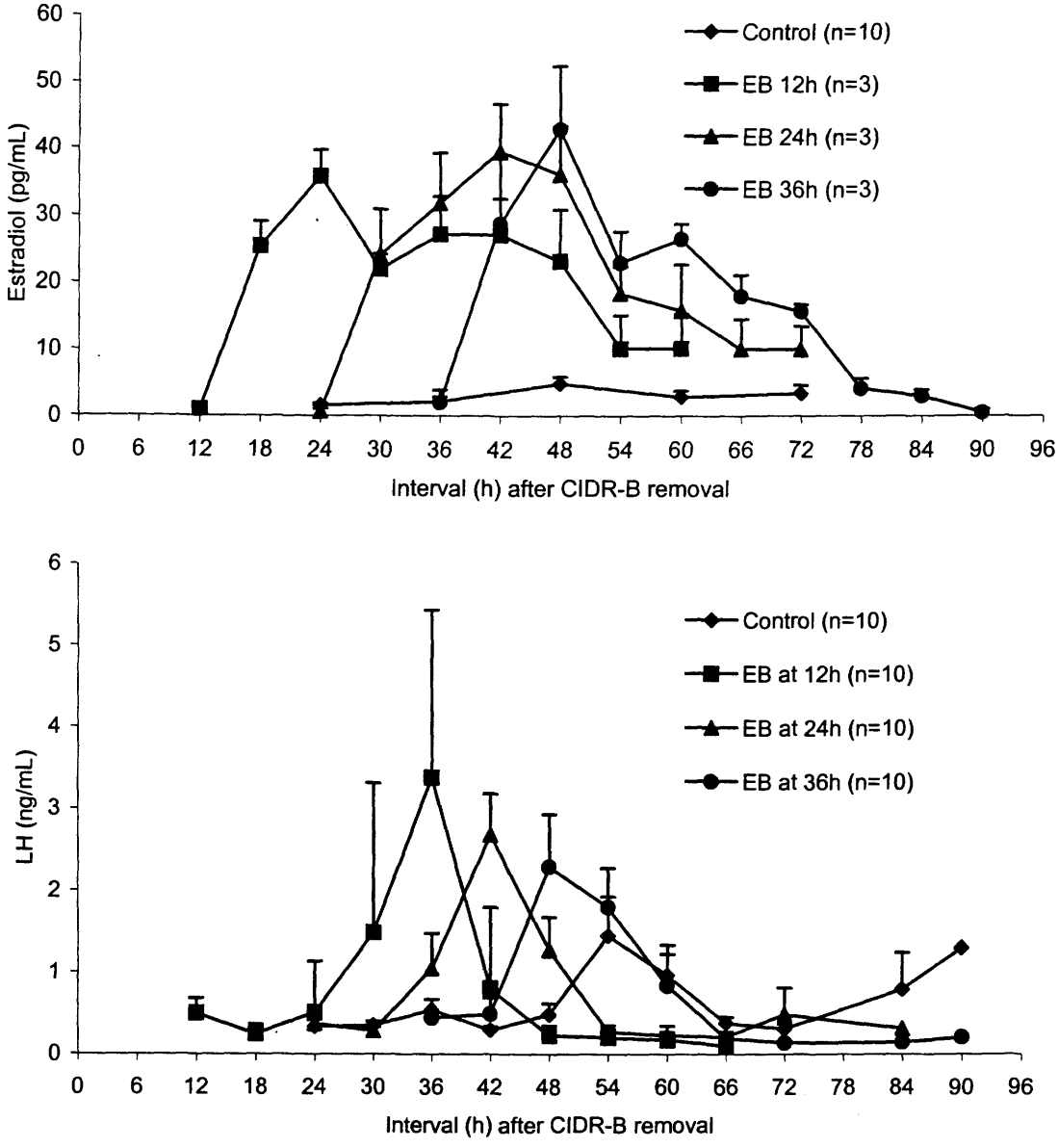


Figure 6.2 Plasma concentrations of estradiol (chart A) and LH (chart B) in Control heifers and heifers given 1.0 mg EB 12, 24 or 36 h after CIDR-B removal (10 heifers per group). Blood samples were collected every 6 h (starting at the time of estradiol treatment). In the Control group, samples were collected for 66 h (starting 24 h after CIDR-B removal).

6.5 Discussion

Treatment with selected doses of E-17β or EB effectively synchronized the emergence of ovarian follicular waves and ovulation in beef cattle. In Experiment 1, the interval from treatment to wave emergence was significantly shorter and more synchronous in cattle given 5 mg E-17β than in other three groups (1 mg E-17β, 1 or 5 mg EB). In previous studies, the administration of 5 mg E-17β in progestin-treated heifers resulted in synchronous emergence of the next follicular wave, on average, 4.3 ± 0.2 days (Bó et al., 1994, 1995b) or 3.4 ± 0.1 days (Martínez et al., 2000) after treatment. In Experiment 2, 1 mg EB consistently induced emergence of a new follicular wave in 3 to 6 days; neither the mean nor the variance were significantly different compared to heifers that received 5 mg E-17β. In dairy cows, administration of 1 mg EB during mid-diestrus was reported to effectively synchronize follicular wave emergence (4.5 ± 0.2 days after treatment) and development of a new ovulatory follicle; however, the range was not reported (Burke et al., 1997a). In contrast, injection of 5 mg EB (Experiment 1) did not consistently induce synchronous follicular wave emergence (range, 4 to 8 days after treatment). In addition, two cows in which follicular wave emergence occurred 2 days before treatment developed a persistent dominant follicle, which apparently delayed emergence of a new follicular wave. In a previous study in beef cows (Caccia and Bó, 1998), treatment with 2.5 mg EB (at CIDR-B insertion) resulted in more synchronous follicular wave emergence (3 to 4 days after treatment) compared to treatment with 5 mg EB (range, 2 to 5 days). It is noteworthy that EB has a longer circulating life-span than E-17β (approximately 36 and 12 h, respectively, O'Rourke et al., 2000; Bó et al., 1994). Therefore, for synchronization of follicular

wave emergence, a lower dose of a long-acting estradiol ester (EB) was approximately as efficacious as a higher dose of E-17β.

In 3/32 (10.3%) and 2/32 (6.2%) of the cattle in Experiments 1 and 2, respectively, estradiol treatment did not induce emergence of a new follicular wave. Bó et al. (1995a) reported similar treatment failures in 3/47 (6.3%) of heifers given 5 mg E-17β and norgestomet implants. It is noteworthy that three of the non-responding cattle in the present studies appeared to be undergoing spontaneous luteal regression (based on the ultrasonographic examination) at the time of estradiol treatment. Burke et al. (1997b) reported that luteolysis within 48 h after treatment with 1 mg EB prevented synchronization of follicular wave emergence in dairy cows. Elevated progestin concentrations may be important for assuring the effectiveness of the estradiol treatment (Bó et al., 1995a); in the absence of elevated progestin concentrations, exogenous estradiol failed to cause regression of the dominant follicle (it resumed growth and emergence of a new follicular wave was delayed). In the present studies, plasma progesterone concentrations during spontaneous luteolysis were apparently insufficient to allow for complete suppression of the growing dominant follicle after estradiol treatment.

There are many factors that may account for treatment failure, most notably the stage of development of the dominant follicle at the time of treatment, but also the stage of development of the CL (and blood progesterone concentrations). For example, when the CL is regressing at the end of the estrous cycle, plasma concentrations of progesterone are decreasing and estradiol concentrations are increasing, LH pulsatility is increasing, and the (preovulatory) dominant follicle has become LH-dependent (Ginther

et el., 1996). We speculated that regression of the preovulatory follicle may require elevated concentrations of progesterone to suppress LH, in addition to estradiol, which would then synchronize the FSH surge and emergence of the new follicular wave. Estradiol treatment alone (in the absence of an endogenous or exogenous source of progestins) resulted in an LH surge; the dominant follicle failed to undergo atresia and continued to grow (Bó et al., 1994). Furthermore, in ovariectomized cows, the injection of progesterone or the presence of a CIDR-B device did not prevent the estradiol-induced LH surge (section 6.0), and presumably this could stimulate growth of an LH-dependent dominant follicle in cattle with very low plasma progesterone concentrations. Concentrations of synthetic progestins normally administered apparently are insufficient to suppress circulating LH concentrations (Kojima et al., 1992), facilitating the continuous growth of the dominant follicle (Savio et al., 1993). If an estradiol/progestin treatment does not suppress the growth of the selected dominant follicle after the onset of regression of the CL (approximately Days 16 to 18 of the bovine estrous cycle), the remaining 2 or 3 days correspond to approximately 10% of the estrous cycle where treatment failures are likely to occur.

For optimal fertility to fixed-time AI, ovulation must occur synchronously (within a few hours) in all animals. Therefore, fixed-time AI necessitates the synchronization of follicular wave emergence, control of follicular growth, and synchronization of ovulation. Increasing concentrations of estradiol (following the decline of progesterone) induce the preovulatory LH surge in normally cyclic cows (Stumpf et al., 1991). In this regard, EB has been used for the synchronization of estrus and ovulation after PGF; 0.4 mg EB 48 h after PGF resulted in 30% more cows detected

in estrus (Dailey et al., 1986) and a more synchronous LH surge than in non-treated (Control) cattle (Welch et al., 1975). In the present study, the interval from CIDR-B removal to estrus and ovulation was highly synchronous in cattle given E-17β or EB after CIDR-B removal.

In a previous study in which the CIDR-B device was left in place for 8 days and follicular wave emergence was induced by treatment with E-17β and progesterone at CIDR-B insertion, ovulation occurred, on average, 82 h (range, 72 to 108 h) after CIDR-B removal (Martínez et al., 2000). In that study, heifers ovulated earlier than the cows in Experiment 1 of this study. To our knowledge, a direct comparison of follicle development in cows and heifers has not been reported. However, these two different studies suggest that differences may exist between these two physiological classes of cattle.

Other factors may affect responses to estradiol treatment after CIDR-B removal. In Experiment 2, a heifer did not respond to E-17β and ovulated 120 h after CIDR-B removal. In Experiment 3, a heifer treated with EB (at 24 h) had a dominant follicle (10 mm in diameter at treatment) that ovulated 108 h after CIDR-B removal, presumably in response to her own endogenous LH surge at 72 h. Burke et al., (2001) reported no effect of the maturity of the follicle on the ovulatory response to EB treatment. However, more information on the factors affecting the interval from estradiol treatment to ovulation is needed.

Concentrations of endogenous estradiol probably contributed to the variation in the interval from CIDR-B removal (or estradiol administration) to ovulation. In

Experiment 3, circulating estradiol concentrations peaked approximately 15 h after EB treatment, with a range of approximately 6 h. In cattle given 10 mg EB during diestrus, plasma estradiol concentrations peaked with a mean (±SEM) concentration of 175.0 ± 29.0 pg/mL at 13 h (Vynckier et al., 1990); this concentration was 10-fold greater than that obtained following administration of 1 mg EB in our study. When 1 mg EB was administered to ovariectomized heifers that received a PRID, plasma estradiol concentrations peaked by 15 h (O'Rourke et al., 2000). In another study, 0.75 mg EB given to intact beef heifers resulted in an increase in plasma estradiol concentrations by 4 h, with a peak (average, 50 pg/mL) at 16 h (coinciding with the LH peak). This demonstrated that lower concentrations of plasma estradiol were needed for the induction of the LH surge under an environment in which progesterone concentrations were baseline (Lammoglia et al., 1998). In experiments involving fixed-time AI in cattle fed MGA for 7 days, estrus and fertility after a single AI were not different after treatment with 0.5 or 1 mg EB on Day 8 (24 h after PGF treatment; Section 8.0). In addition, an injection of 1 mg EB 24 h after CIDR-B removal in ovariectomized cows (Section 5.0) resulted in an average peak concentration of estradiol of 15 pg/mL, which was sufficient to trigger the LH surge 6 h later.

Plasma LH concentrations in heifers given EB 12 or 24 h after CIDR-B removal, declined slightly by 6 h and then increased, reaching maximal values between 20 and 25 h after estradiol treatment. Similar hormonal patterns following estradiol treatments have been reported previously and have been attributed to a biphasic effect of estradiol on plasma LH concentration which was described in ovariectomized beef heifers (Bolt et al., 1990). In that study, plasma LH concentrations were lower at 4, 8 and 12 h after

treatment with 10 mg E-17β but were higher at 20, 24 and 28 after estradiol treatment than that of control animals. In experiments using ovariectomized cows (Section 5.0), plasma LH concentrations declined transiently (6 h after E-17β treatment), followed by an increase 18 to 24 h after treatment. In the present study, low doses of EB were used; therefore, other factors (e.g. progesterone decline) probably influenced LH release.

In Experiment 3, it appeared that there was an effect of plasma progesterone concentrations on the timing of LH release. In heifers given 1 mg EB 12 h after CIDR-B removal, the interval from EB treatment to LH surge (25 h) was longer than treatment at 36 h (15 h) after CIDR-B removal. The interval to the LH surge also tended to be longer in heifers treated with EB at 12 h than in heifers that received EB at 24 h (20 h). Although progesterone concentrations decreased to less than 1 ng/mL at 24 h in all treatment groups, blood sampling was not frequent enough to determine when progesterone concentrations reached baseline. The presence of progesterone may have affected the timing of estradiol-induced LH release. There may be other factors that affected the interval to LH release and ovulation, such as stage of dominant follicle growth and proximity to the selection of the dominant follicle at the time of progesterone decline.

The interval from CIDR-B removal to ovulation was shorter in heifers treated with EB 12 h after CIDR-B removal (64 h) than in heifers treated with EB at 24 (82 h) or 36 h (78 h). These two latter intervals were comparable to those observed in Experiment 2 for E-17β (76 h) or EB (77 h) given 24 h after CIDR-B removal. If ovulation is expected approximately 75 to 80 h after CIDR removal, fixed-time AI should be done about 28 to 34 h after estradiol treatment (52 to 58 h after CIDR

removal) in beef cattle. In similar studies, EB was administered 30 h after progesterone insert removal and resulted in pregnancy rates greater than 50% following fixed-time AI 28 to 30 h later (Bridges et al., 1999). Once the estradiol-induced endogenous LH surge occurred, heifers in Experiment 3 ovulated with no significant differences among groups. Although there was a numerical difference in the mean interval from LH peak to ovulation between heifers in the Control group vs those in the group treated with estradiol 24 h after CIDR-B removal (24 and 37 h, respectively), this difference was attributed to the ultrasonographic examinations which were performed at 12-h intervals.

Both E-17β and EB effectively synchronized follicular wave emergence and ovulation. The favored dose for the synchronization of follicular wave emergence was 5 mg E-17β or 1 mg EB. Time of ovulation following CIDR-B removal was hastened in all EB-treated groups as compared to Controls, and was significantly earlier in heifers treated with EB 12 h after CIDR-B removal than in those treated with EB at 24 or 36 h after CIDR-B removal. However, EB treatment administered at 24 h consistently synchronized ovulation and, allowed the preovulatory follicle to mature for another 12 h compared to treatment at 12 h. For fixed-time AI, administration of EB 24 h after CIDR-B removal reduced the risk of early ovulations, which would adversely affect fertility following fixed-time AI. Finally, from a management perspective, 24-h intervals between treatments would enable treatments to be given during a consistent time, i.e., in the morning.

7.0 ESTRUS SYNCHRONIZATION AND PREGNANCY RATES IN BEEF CATTLE GIVEN CIDR-B, PROSTAGLANDIN AND ESTRADIOL OR GNRH

7.1 Abstract

Two experiments were conducted to determine estrous response and pregnancy rate in beef cattle given a controlled internal drug release (CIDR-B) device plus prostaglandin F2α (PGF) at CIDR-B removal, and estradiol or gonadotropin releasing hormone (GnRH). In Experiment 1, crossbred beef heifers received a CIDR-B device and 1 mg estradiol benzoate (EB) plus 100 mg progesterone (E+P group; n = 41), 100 μg gonadorelin (GnRH group; n = 42) or no further treatment (Control group; n = 42) on Day 0. On Day 7, CIDR-B devices were removed and heifers were treated with PGF. Heifers in the E+P group were given 1 mg EB 24 h after PGF and were inseminated 30 h later. Heifers in the GnRH group were given 100 μg GnRH 54 h after PGF and concurrently inseminated. Control heifers were inseminated 12 h after onset of estrus. The estrus rate was lower ($P < 0.01$) in the GnRH group (54.7%) than in either the E+P (100%) or Control (83.3%) groups. The mean interval from CIDR-B removal to estrus was shorter ($P < 0.01$) and less variable ($P < 0.01$) in the E+P group than in the GnRH or Control groups. Pregnancy rate in the E+P group (75.6%) was higher ($P < 0.01$) than in the GnRH (47.6%) or Control (38.1%) groups.

In Experiment 2, 84 cows were treated similarly to the E+P group in Experiment I. Cows received 100 mg progesterone and either 1 mg EB or 5 mg estradiol-17β (E-17β) on Day 0 and either 1 mg of EB or 1 mg of E-17β on Day 8 (24 h after CIDR-B removal) in a 2x2 factorial layout and were inseminated 30 h later. There were no differences among groups for estrus rates or conception rates. The mean interval from CIDR-B removal to estrus was 44.2 ± 1.2 h. Conception rates were 66.7, 61.9, 52.4, and 71.4% in Groups E-17β/E-17β, E-17β/EB, EB/E-17β and EB/EB, respectively. In cattle given a CIDR-B device and estradiol plus progesterone, treatment with either EB or E-17β effectively synchronized estrus and resulted in acceptable conception rates to fixed-time artificial insemination.

7.2 Introduction

Recent approaches to estrus synchronization and fixed-time artificial insemination (AI) involve control of the luteal phase (endogenous and/or exogenous source of progestins), synchronization of follicular wave emergence, and synchronization of the preovulatory LH surge and ovulation (Adams, 1998). One protocol employing this approach is the Ovsynch regimen, consisting of two gonadotropin releasing hormone (GnRH) treatments (8 to 9 d apart), prostaglandin $F_{2\alpha}$ (PGF) treatment 30 to 48 h before the second GnRH treatment, and timed AI 0 to 24 h after the second GnRH treatment (Wiltbank, 1997). The first GnRH treatment usually increases peripheral progesterone concentrations by inducing ovulation of a dominant

follicle (Martínez et al., 1999) and thereby synchronizes emergence of a new follicular wave. The second GnRH treatment synchronizes the preovulatory luteinizing hormone (LH) surge and ovulation. However, 8.7% and 11.8% of cows have been reported to be detected in estrus between the first GnRH and PGF injections for 6 and 7 d programs, respectively (Roy and Twagiramungu, 1999). Exogenous progestin treatment would be expected to minimize the expression of estrus between the first GnRH and PGF treatments. In that regard, short-term progestin treatments have been successfully used to synchronize estrus (Macmillan and Peterson, 1993). Furthermore, estrogens have been shown to synchronize both follicle wave emergence (Bó et al., 1995 a, b; Caccia and Bó, 1998) and the preovulatory LH surge (Lammoglia et al., 1998) and may be used instead of GnRH for these purposes.

The purpose of the present study was to determine the estrous response and pregnancy rate in beef cattle given a controlled internal drug release (CIDR-B) device and PGF at CIDR-B removal and estradiol or GnRH to induce follicle wave emergence and then to synchronize ovulation for fixed-time AI.

7.3 Materials and Methods

7.3.1 Experiment 1

Postpubertal (ultrasonographic detection of a corpus luteum), cross-bred (Simmental-Angus-Gelbvieh) beef heifers (n = 125) were housed outdoors in feedlot pens at a farm near Saskatoon (Saskatchewan) and fed barley silage, with free access to

water. Heifers received a CIDR-B device (Vetrepharm Canada Inc, Belleville, Ontario, Canada) at random stages of the estrous cycle (device insertion = Day 0). Heifers were randomly allocated to receive: 1 mg of estradiol benzoate (EB) and 100 mg of progesterone (Sigma Chemical Co, St Louis, Missouri, USA) dissolved in 2 mL of canola oil (E+P group; n = 41); 100 μg gonadorelin acetate (Cystorelin, Merial Canada Inc, Victoriaville, Québec, Canada; GnRH group; n = 42); or no further treatment (Control group; n = 42). On Day 7, CIDR-B devices were removed and heifers were given 500 μg cloprostenol (PGF; Estrumate, Schering-Plough Animal Health, Pointe Claire, Québec, Canada). Heifers in the E+P group were given 1 mg EB 24 h after PGF and were inseminated 30 h later (54 h after PGF). Heifers in the GnRH group were given 100 μg gonadorelin 54 h after PGF and concurrently inseminated. Control heifers were inseminated 12 h after onset of estrus. All hormone solutions were injected i.m. Estrus detection devices (Kamar Heatmount Detector; Kamar Inc, Steamboats Springs, Colorado, USA) were glued onto the sacral regions of the heifers at the time of CIDR-B removal. Heifers were observed for signs of mounting behaviour and the mount detectors were examined for evidence of activation (red color) twice daily for 5 d after CIDR-B removal. The onset of estrus was defined as the first observation period at which the detector device had been activated or the heifer was observed in standing estrus. Pregnancy diagnosis was conducted 30 d after CIDR-B removal with a real-time, B-mode scanner (Aloka SSD-500, Instruments for Science and Medicine Inc, Edmonton, Alberta, Canada) equipped with a 7.5 MHz linear-array transducer.

Estrus rate was defined as the proportion of heifers detected in estrus. Conception rate was defined as the percentage of inseminated heifers that became pregnant and pregnancy rate was defined as the percentage of the total number of heifers in the group that became pregnant. Mean and standard error of the mean (SEM) were used to describe data. For the interval from PGF treatment to estrus, analysis of variance and Bartlett's test were used to compare means and variances, respectively. If there was a significant effect of group on means, differences were located with a least significant difference (LSD) multiple range test. Chi-square analyses were used to detect differences among treatments for estrus, conception and pregnancy rates. All statistical analyses were conducted with a commercial program for statistical analysis (Statistix Student Version, version 2.0, Analytical Software, Tallahassee, Florida, USA).

7.3.2 Experiment 2

Eighty-four, suckled crossbred beef cows that were at least 50 d postpartum and kept on pasture of native grass at the Animal Disease Research Institute at Lethbridge (Alberta) were treated similarly to the E+P group in Experiment I. Cows received 100 mg progesterone and either 1 mg EB or 5 mg estradiol-17β (E-17β; Sigma Chemical Co) on Day 0 and either 1 mg of EB or 1 mg of E-17β on Day 8 (24 h after CIDR-B removal and PGF treatment) in a 2x2 factorial design. On Day 7, CIDR-B devices were removed and cows were given PGF. Estrus detection was done by twice-daily observations. Cows were artificially inseminated 30 h after the second estradiol injection (54 h after PGF treatment and CIDR-B removal), whether they showed estrus or not. Pregnancy

diagnosis was conducted by rectal palpation 45 d after PGF treatment. Statistical analyses were conducted as described in Experiment 1.

The protocols for these experiments were approved by University of Saskatchewan Animal Care Committee.

7.4 Results

7.4.1 Experiment I

Results are summarized in Table 1. The estrus rate was significantly different ($P < 0.01$) between the GnRH group (54.7%), and the E+P group (100%) and the Control group (83.3%). The mean interval from CIDR-B removal to estrus was shorter ($P < 0.01$) and less variable ($P < 0.01$) in the E+P group (48.6 ± 0.4 h) than in either the GnRH (63.7 ± 3.5 h) or Control (65.1 ± 3.0 h) groups. Conception rate in the E+P group (75.6%) was higher ($P < 0.01$) than in both the GnRH (47.6%) and Control (38.1%) groups (35 of 42 heifers in the Control group were observed in estrus and inseminated, and 16 became pregnant).

Table 7.1 Estrus, conception and pregnancy rates in Control heifers or those treated with estradiol benzoate and progesterone (E+P) or GnRH in a CIDR-B-based estrus synchronization program with PGF at CIDR-B removal.

End points	Control	E+P	GnRH
No. of heifers	42	41	42
PGF to estrus			
Mean (h)	65.1 [b]	48.6 [a]	63.7 [b]
SEM	3.0 [y]	0.4 [x]	3.5 [y]
Range (h)	48 to 120	48 to 60	48 to 108
Estrus rate (%)	35 (83.3)[c]	41 (100)[a]	23 (54.7)[b]
Conception rate (%)	16/35 (45.7)[b]	31 (75.6)[a]	20 (47.6)[b]
Pregnancy rate (%)	16 (38.1)[b]	31 (75.6)[a]	20(47.6)[b]

[ab] Means or percentages with different superscripts were different ($P < 0.01$).
[xy] Variances with different superscripts were different ($P < 0.01$).
SEM = standard error of the mean.

7.4.2 Experiment 2

Although some cows lost their CIDR-B devices (11/84, 13%; no differences among groups), they were inseminated at a fixed-time along with those that retained their CIDR-B devices. Results are shown in Table 2. There were no significant differences among groups for estrus rates or conception rates ($P = 0.67$ and $P = 0.61$, respectively). Estrus rate for all cows combined (69/84; 82.1%) was not different ($P = 0.70$) from only those with a retained CIDR-B (60/74; 81.1%). Similarly, there was no difference ($P = 0.82$) in conception rates between all cows combined and cows with a retained CIDR-B (53/84, 63.1% vs. 46/75, 61.3%).

Table 7.2 Response of cows treated with a CIDR-B device and given estradiol-17β (E-17β) or estradiol benzoate (EB) plus 100 mg progesterone at CIDR-B insertion and either E-17β or EB 24 h after PGF treatment and CIDR-B removal.

End point	E-17β/E-17β	E-17β/EB	EB/E-17β	EB/EB
No. of cows	21	21	21	21
Estrus rate (%)				
All cows	76.2 (16/21)	90.4 (19/21)	81.0 (17/21)	81.0 (17/21)
Cows with retained CIDR-B	70.6 (12/17)	88.9 (16/18)	80.0 (16/20)	78.9 (15/19)
PGF to estrus				
Mean (h)	41	46	44	46
SEM		1.3	1.1	1.3
Range	36 to 48	36 to 48	36 to 48	36 to 48
Conception rate (%)[a]				
All cows	66.7 (14/21)	61.9 (13/21)	52.4 (11/21)	71.4 (15/21)
Cows with retained CIDR-B	64.7 (11/17)	61.1 (11/18)	50.0 (10/20)	70.0 (14/20)

[a] Fixed-time insemination at 54 h after PGF treatment and CIDR-B removal.
SEM = standard error of the mean.
No significant differences were found among groups for estrus (P = 0.67) or pregnancy rates (P = 0.61).

7.5 Discussion

In Experiment 1, all heifers in the E+P group were detected in estrus and 75.6% became pregnant to a fixed-time AI. In the GnRH group, the conception rate to a fixed-time AI (47.6%) was acceptable, but the estrus rate (54.7%) was approximately half of that in the E+P group. In another study, in which GnRH was given 7 d before PGF treatment, behavioural estrus (after PGF treatment) was detected in 51.3% of cows (Roy and Twagiramungu, 1999). Treatment with GnRH induces LH release which may cause premature luteinization of ovarian follicles (thereby decreasing peripheral estrogen concentrations) or ovulation may be induced before peripheral estrogen concentrations peak, accounting for the lower rate of estrus detection. The principal purpose of detecting estrus is to determine the appropriate time at which to inseminate in anticipation of ovulation. However, estrus detection, and indeed whether or not estrus is expressed, is unimportant if ovulation is synchronized in all or most cattle. Conception rate in the Control group was nearly identical to that in the GnRH group; however, due to a moderate rate of estrus detection, some heifers were not inseminated and the overall pregnancy rate was somewhat less than expected. Obviously, when cattle are only inseminated following detection of estrus, the combination of a moderate estrus rate and a moderate conception rate results in a relatively low pregnancy rate.

In Experiment 2, EB and E-17β were equally effective in inducing a high rate of estrus and pregnancy. Historically, estrogens have been given at the beginning of a progestin treatment regimen to induce luteolysis (via induction of PGF release; Wiltbank and Kasson, 1968). However, the high rates of estrus and pregnancy in cows that lost

their CIDR-B devices in this experiment suggests that these doses (and/or forms) of estradiol did not induce luteolysis. More recently, it has been demonstrated that estrogen treatment causes regression of antral follicles and synchronizes emergence of a new follicular wave (Bó et al., 1995a). Further, it has been shown that, in the absence of elevated progestin concentrations, estrogen treatment induces an LH surge, resulting in transient or incomplete suppression of the dominant follicle and delayed emergence of the next follicular wave (Bó et al., 1994). Therefore, on the possibility of encountering cows without corpus luteum in a group of randomly cycling cows, we have consistently included progesterone with the first estrogen treatment. Estrogen treatment has been given after PGF treatment to increase the proportion of cattle in estrus and the synchrony of estrous behaviour and ovulation (Dailey et al., 1986). In other studies, when 0.5 or 1.0 mg EB was given after CIDR-B removal, the proportion of heifers detected in estrus was higher than in Control heifers that did not receive estradiol (Hanlon et al., 1996, Rasby et al., 1998). In another study (Lammoglia et al., 1998), treatment with EB (0.38 and 1.0 mg in heifers and cows, respectively) 24 to 30 h after progesterone removal, effectively induced estrus (estrus rate, 86% and 100%, respectively). Furthermore, estrogen treatment in the present study appeared to induce an estrus more characteristic of a spontaneous estrus (considerable clear mucus discharge and a cervical canal that was patent) compared with that in the GnRH treatment group.

Two doses of GnRH are given 7 d apart in the Ovsynch regimen and PGF is given just before the second injection (Pursley et al., 1995). The first injection has been reported to induce ovulation (18/20 cows and 13/24 heifers responded by ovulating) or luteinization, followed by the emergence of a new follicular wave in 2.1 ± 0.31 or 1.5 ±

0.47 days in cows and heifers, respectively. The second injection of GnRH is given to induce an LH surge and thereby synchronize ovulation. All 20 cows and 18 of 24 heifers ovulated between 24 and 32 h after the second injection of GnRH, suggesting that the Ovsynch regimen may not be as efficacious in heifers (Pursley et al., 1995). It seems that heifers that failed to ovulate following the second GnRH treatment were in metestrus or early diestrus and did not undergo luteolysis in response to the PGF treatment. Therefore, if the first GnRH treatment does not induce ovulation and emergence of a new follicular wave, ovulation of a dominant follicle following PGF treatment and a second injection of GnRH may be poorly synchronized. Indeed, Wiltbank (1997) observed that up to 20% of heifers were detected in estrus before the PGF treatment in Ovsynch programs. In a recent study (Martínez et al., 1999), GnRH treatment induced ovulation in 56% of heifers treated during the growing, static or regressing phases of the dominant follicle of the first follicular wave; it is noteworthy that emergence of a new follicular wave occurred only when treatment caused ovulation. We have also shown that insertion of a CIDR-B device prevents the expression of estrus between the first injection of GnRH and PGF treatment, and pregnancy rates to a fixed-time insemination were higher when a CIDR-B device was used in an Ovsynch program in heifers (Section 10.0).

Estrogens in combination with progesterone administered at the beginning of a CIDR-B treatment resulted in synchronous emergence of a new follicular wave in both heifers and cows (Bó et al., 1996). The estrogens used in the present study (E-17β and EB) have a shorter half-life in circulation than either estradiol valerate (commonly included in norgestomet-based estrus synchronization protocols; Bó et al., 1993) or

estradiol cypionate (Thundathil et al., 1997). Treatment with the latter two formulations of estradiol induced emergence of a new follicular wave at inconsistent and prolonged intervals. It is noteworthy that E-17β has a shorter circulating half-life than does EB. Following treatment with 5 mg E-17β, peripheral estradiol concentrations returned to baseline within 48 h (Bó et al., 1994), whereas treatment with 5 or 10 mg of EB resulted in elevated concentrations of serum estradiol for approximately 72 and 96 h, respectively (Vynckier et al., 1990). Follicular wave emergence has been reported to occur 4.3 d after treatment of progestagen-treated cattle with 5 mg of E-17β (Bó et al., 1995b) and 5.4 d after treatment with 5 mg of EB (Bó et al., 1996).

Subsequent to the completion of these studies, Bridges et al (1999) reported an experiment utilizing 139 lactating postpartum beef cows that were given a CIDR-B device and allocated into three treatment groups to receive 2 mg EB at the time of device insertion (Groups 1 and 2) or no EB (Group 3) at CIDR-B insertion. The CIDR-B devices were subsequently removed after 7 d (Group 1) or 5 d (Groups 2 and 3) and all cows were given 25 mg PGF at that time plus 1 mg EB 30 h after device removal and were inseminated 28 to 30 h later (58 to 60 h after device removal). Estrus rates were 93, 87 and 81% and pregnancy rates were 60, 50 and 51% for cows in Groups 1, 2, and 3, respectively. These results are comparable with those reported in the present study suggesting that there may be considerable flexibility as to when the CIDR-B device may be removed without interfering with pregnancy rates to fixed-time insemination.

In the present studies, both E-17β and EB were highly efficacious in synchronizing estrus, and presumably ovulation, in a CIDR-B-based, fixed-time AI program in cattle. Further studies are needed to confirm the necessity of synchronizing

follicular wave emergence and to precisely document the effects of length of CIDR-B treatment and intervals to a second estradiol treatment on the time of ovulation in order to optimize the time of insemination and pregnancy rates.

8.0 THE USE OF GnRH OR ESTRADIOL TO FACILITATE FIXED-TIME INSEMINATION IN AN MGA-BASED SYNCHRONIZATION REGIMEN IN BEEF CATTLE

8.1 Abstract

Two experiments were conducted to compare pregnancy rates when GnRH or estradiol were given to synchronize ovarian follicular wave emergence and ovulation in an MGA-based estrus synchronization program. Crossbred beef cattle were fed melengestrol acetate (MGA, 0.5 mg/day) for 7 days (designated Days 0 to 6, without regard to stage of the estrous cycle) and given cloprostenol (PGF; 500 μg im) on Day 7. In Experiment 1, lactating beef cows (n = 140) and pubertal heifers (n = 40) were randomly allocated to three groups to receive 100 μg gonadorelin (GnRH), 5 mg estradiol-17β and 100 mg progesterone (EP) in canola canola oil or no treatment (Control) on Day 0. All cattle were observed for estrus every 12 hours from 36 to 96 hours after PGF. Cattle in the GnRH group that were detected in estrus 36 or 48 hours after PGF were inseminated 12 hours later; the remainder were given 100 μg GnRH i.m. 72 hours after PGF and concurrently inseminated. Cattle in the EP group were randomly assigned to receive either 0.5 mg or 1.0 mg estradiol benzoate (EB) in 2 mL canola canola oil i.m. 24 hours after PGF and were inseminated 30 hours later. Cattle in the Control group were inseminated 12 hours after the first detection of estrus; if not in estrus by 72 hours after PGF, they were given 100 μg GnRH i.m. and concurrently inseminated. In the absence

of significant differences (P>0.1), all data for heifers and for cows were combined and the 0.5 and 1.0 mg EB groups were combined into a single estradiol group. Estrus rates were 57.6, 57.4 and 60.0% for the GnRH, EP and Control groups, respectively (P = 0.95). The mean (± SD) interval from PGF treatment to estrus was shorter (P < 0.001) and less variable (P < 0.001) in the EP group (49.0 ± 6.1 h) than in either the GnRH (64.2 ± 15.9 h) or Control (66.3 ± 13.3 h) groups. Overall pregnancy rates were higher (P < 0.005) in the GnRH (57.6%) and EP (55.7%) groups than in the Control group (30.0%) as were pregnancy rates to fixed-time AI (47.5, 55.7 and 28.3%, respectively). In Experiment 2, 122 crossbred beef heifers were treated with melengestrol acetate (MGA) from Day 0 (beginning of the experiment to Day 6 and given either 100 μg GnRH or 2 mg EB and 50 mg progesterone in canola oil on Day 0. Heifers subsequently received either 100 μg GnRH 36 h after PGF (given on Day 7) and inseminated 14 h later or 1 mg EB i.m. 24 h after PGF and inseminated 28 h later in a 2 x 2 factorial design. Pregnancy rates were not significantly different among groups (41.9, 32.2, 33.3 and 36.7% in GnRH/GnRH, GnRH/EB, EB/GnRH and EB/EB groups, respectively). In conclusion, GnRH or estradiol given to synchronize ovarian follicular wave emergence and ovulation in an MGA-based synchronization regimen resulted in acceptable pregnancy rates to fixed-time AI.

8.2 Introduction

Melengestrol acetate (MGA), a synthetic progestin, has been used in various regimens to synchronize estrus in cattle (Patterson et al., 1989; Odde, 1990). However, when MGA is fed in the absence of a functional corpus luteum (CL), a dominant follicle persists and fertility at the ensuing estrus is reduced (Custer et al., 1994; Patterson et al., 1989). The development of persistent follicles may be prevented by strategically synchronizing follicular wave emergence; either GnRH or estradiol-17β (E-17β) and progesterone have been given on the first day of a short-term (7- or 8-day) MGA regimen to synchronize emergence of a new wave (Kastelic et al., 1996; Thundathil et al., 1999). However, these regimens required detection of estrus because ovulation was not synchronized. In other programs, treatments to synchronize the preovulatory gonadotropin surge have resulted in relatively synchronous ovulation, facilitating artificial insemination (AI) at a fixed time. For example, in the Ovsynch regimen (Pursley et al., 1995), two doses of GnRH are given approximately 9 days apart (with PGF given 7 days after the first dose of GnRH). The first dose of GnRH is intended to cause ovulation or atresia of large antral follicles, with a new follicular wave emerging approximately 2 days later (Twagiramungu et al., 1995). The second dose of GnRH is given to induce LH release and synchronous ovulation (Pursley et al., 1995). In cyclic heifers that had a progesterone-releasing device (CIDR-B) in their vagina for 7 days and given PGF at CIDR-B removal, estradiol treatment 24 to 30 hours later effectively synchronized estrus with an acceptable pregnancy rate (52%; Lammoglia et al., 1998). Similar pregnancy rates (65%) have been achieved after fixed-time AI with exogenous

progesterone delivered by intravaginal CIDR-B devices in heifers assigned to an Ovsynch program (Section 10.0).

Two experiments were conducted to examine the utility of a short-term MGA treatment protocol in a fixed-time AI program in beef cattle when GnRH or estradiol were given to synchronize ovarian follicular wave emergence and ovulation.

8.3 Materials and Methods

8.3.1 Experiment 1

Crossbred (predominantly Angus and Hereford) lactating beef cows (n = 140) and pubertal heifers (n = 40) were used. At the start of the experiment, cows were between 30 and 70 days postpartum and had a body condition score of 1.5 to 3.0 (scale of 1 to 5). The heifers had a body condition score of 3.0 to 3.5 and a CL was detected by transrectal ultrasonography. The experimental design is summarized in Figure 1. All cattle were fed 0.5 mg/head/day MGA (Pharmacia Animal Health, Orangeville, Ontario, Canada) for 7 days (designated Days 0 to 6), without regard to the stage of the estrous cycle. On Day 0, cattle were randomly assigned to three groups to receive an intramuscular (im) injection of 100 μg gonadorelin (GnRH group; n = 59; Cystorelin, Merial Canada Inc., Victoriaville, Quebec, Canada) or 5 mg estradiol-17β (E-17β) plus 100 mg progesterone (both from Sigma Chemical Co., St. Louis, Missouri, USA) in 2 mL canola canola oil (EP group; n = 61) or no treatment (Control group; n = 60). On Day 7, all cattle were given an i.m. injection of 500 μg of cloprostenol (PGF; Estrumate,

Schering Plough Animal Health, Pointe-Claire, Quebec, Canada). In a randomly selected sub-sample of cattle (11 cows and 7 heifers in each treatment group), a transrectal ultrasound examination was done at the time of PGF treatment and the diameter of the largest follicle was recorded. All cattle were observed for estrus (defined as standing when mounted by a herdmate) every 12 hours from 36 to 96 hours after PGF. Cattle in the GnRH group that were detected in estrus 36 or 48 hours after PGF were inseminated 12 hours later and considered not pregnant to the fixed-time AI; the remainder were given 100 μg GnRH i.m. 72 hours after PGF and concurrently inseminated. Cattle in the EP group were randomly assigned to receive either 0.5 mg or 1.0 mg estradiol benzoate (EB; Sigma Chemical Co., St. Louis, Missouri, USA) in 2 mL canola canola oil i.m. 24 hours after PGF and were inseminated 30 hours later. Cattle in the Control group were inseminated 12 hours after the first detection of estrus; if not in estrus by 72 hours after PGF, they were given 100 μg GnRH i.m. and concurrently inseminated. In each group, cattle were defined as having a synchronous estrus if they were detected in estrus < 24 hours before or after fixed-time AI was planned (i.e., 54 h after PGF in the EP group and 72 hours after PGF in the other two groups); cattle in estrus ≥ 24 hours before or after fixed-time insemination was planned were defined as having asynchronous estrus.

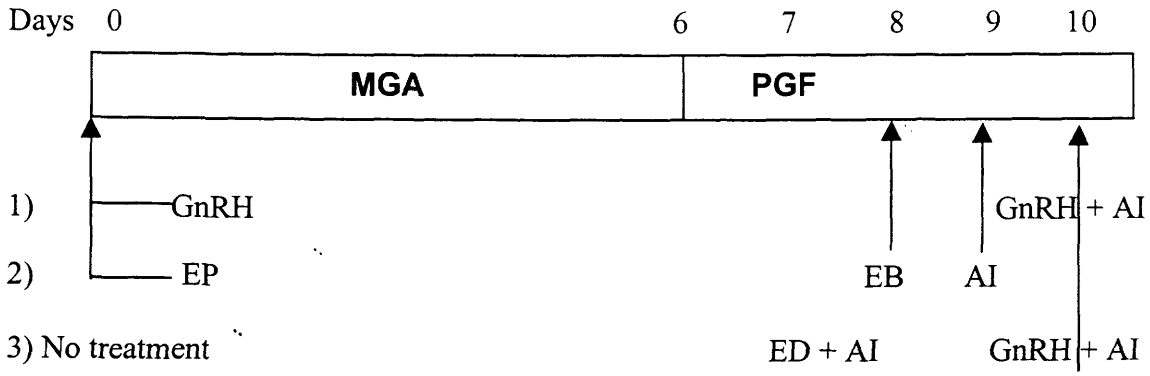


Figure 8.1. Schematic diagram of treatments in Experiment 1. All treatment groups received MGA from Days 0 to 6 and PGF on Day 7. The three treatment groups are each on a separate row, with arrows indicating when specific activities occurred.
Note: All groups were estrus detected and cattle in Groups 1 and 3 were inseminated accordingly if they displayed asynchronous estrus with respect to the fixed-time AI at 72 hours after PGF treatment; all animals in the EP group were fixed-time inseminated 54 hours after PGF treatment.

MGA = melengestrol acetate
PGF = prostaglandin $F_{2\alpha}$
GnRH = 100 μg gonadorelin
EP = 5 mg estradiol-17β plus 100 mg progesterone
EB = 0.5 or 1 mg estradiol benzoate
ED = estrus detection
AI = artificial insemination

Any animal that had an asynchronous estrus was bred approximately 12 hours later and was considered nonpregnant to fixed-time insemination. Any animal that received a fixed-time insemination and subsequently was detected in estrus ≥24 hours later was rebred and considered asynchronous.

In all cattle, pregnancy was confirmed by a transrectal ultrasound examination conducted approximately 30 days after AI. All transrectal ultrasound examinations were done by a single operator using a real-time, B-mode scanner with a 7.5 MHz linear-array transducer (Aloka SSD-500, ISM Inc, Edmonton, AB, Canada).

Estrus rate (synchronous and asynchronous) was the proportion of cattle detected in standing estrus. Conception rate (proportion of inseminated cattle that were pregnant) was calculated for synchronous estrus, asynchronous estrus, and no estrus. Pregnancy rate (proportion of all cattle that were pregnant) was calculated for fixed-time AI and overall (pregnant to fixed-time AI plus AI following asynchronous estrus).

Mean and standard error of the mean (SEM) were used to describe data. Two-way Analysis of Variance was used to detect the effect of age (cows versus heifers) and treatment group (and their interaction) on the diameter of the largest follicle at the time of PGF treatment and on the interval from PGF treatment to estrus; differences among means were compared by a Least Significant Difference test. A Bartlett's test was used to compare variation in the interval from PGF treatment to estrus among groups. Chi-square analyses were used to detect differences between cows and heifers and among treatments for estrus, conception and pregnancy rates. All statistical analyses were conducted with the Statistical Analysis System (SAS, 1990).

The protocols for these experiments were approved by University of Saskatchewan Animal Care Committee.

8.3.2 Experiment 2

Crossbred (Hereford and Angus) beef heifers (n = 122; approximately 14 to 16 months of age, body condition score 2.5 to 3.0 and 393.5 ± 2.9 kg) were used. For several weeks prior to the start of the experiment, these heifers were fed a high-energy diet (principally grain), however, they were fed a mainly roughage diet, from 2 weeks before initiating treatments to the end of the experiment. All heifers were given MGA and PGF as described in Experiment 1 and were randomly allocated to the following four groups in a 2 x 2 factorial design: GnRH versus EB (and progesterone) on Day 0 and GnRH versus EB after PGF. On Day 0, half of the heifers were given 100 μg of GnRH i.m. and the remainder were given 2 mg EB plus 50 mg progesterone i.m. (in 2 mL of canola oil). On Day 8 (approximately 24 hours after PGF treatment), half of the heifers in each group were given 1 mg of EB i.m. and were inseminated 28 hours later. In the evening of Day 8 (36 hours after PGF), the remaining heifers in each group received 100 μg GnRH im; these heifers were inseminated in the morning of Day 9 (14 hours later; 50 hours after PGF). Observations for estrus were not done. Pregnancy was confirmed by transrectal ultrasonography conducted approximately 30 days after breeding. Chi-square analysis was used to compare pregnancy rates among treatments.

8.4 Results

8.4.1 Experiment 1

Combined for all three treatment groups, there was no difference between cows and heifers for estrus rates (57.1 versus 62.5%, respectively; $P < 0.6$) or pregnancy rates (49.3 versus 42.5%, $P < 0.5$). Furthermore, within each treatment group, there were no significant differences ($P>0.2$) between cows and heifers for estrus rates or pregnancy rates. In the EP group, neither estrus nor pregnancy rates were significantly affected by the dose of EB (0.5 versus 1.0 mg). Therefore, all estrus and pregnancy data were combined without regard for animal age (cows versus heifers) or dose of EB.

Mean (± SEM) diameter of the largest follicle at PGF treatment was not different between cows and heifers (11.7 ± 0.3 mm versus 12.5 ± 0.2 mm, respectively, $P < 0.2$) and the age by treatment interaction was not significant ($P < 0.9$). However, there was an effect of treatment ($P < 0.0001$); follicle diameters were 10.5 ± 0.3 (range, 4 to 13), 12.2 ± 0.2 (range, 10 to 14) and 13.6 ± 0.3 mm (range, 8 to 16) for the EP, GnRH and Control groups, respectively (each treatment was different from the other, $P < 0.05$). Estrus rates were not different ($P = 0.95$) among treatment groups (overall mean, 58.3%; Table 8.1). There was an effect of treatment group on the interval from PGF treatment to estrus ($P < 0.0001$) but neither the effect of age nor the interaction were significant ($P > 0.25$). The interval from PGF treatment to estrus was shorter ($P < 0.001$) and less variable ($P < 0.001$) in the EP group (49.0 ± 0.8 h) than in either the GnRH (64.2 ± 2.0 h) or Control (66.3 ± 1.7 h) groups. Pregnancy rates (to both fixed-time AI and overall) were higher ($P < 0.005$) in the GnRH and EP groups than in the Control group (Table

8.1). Within each treatment group, there was no significant difference in conception rate between cattle that had a synchronous estrus versus those that were not detected in estrus.

Table 8.1 Interval from PGF treatment to estrus, and rates of estrus, conception and pregnancy in beef cattle fed MGA for 7 days (Experiment 1).

End point	GnRH	EP	Control
No. cattle	59	61	60
PGF to estrus (hours)			
Mean	64.2[a]	49.0[b]	66.3[a]
SEM	2.0[a]	0.8[b]	1.7[a]
Estrus rate (%)	57.6	57.4	60.0
Synchronous estrus			
No.	22	35	28
Conception rate (%)	68.2[a]	62.5[a]	35.7[b]
No estrus detected			
No.	25	26	24
Conception rate (%)	52.0	46.1	29.2
Asynchronous estrus			
No.	12	--	8
Conception rate (%)	50.0	--	12.5
Pregnancy rate (%)			
Fixed-time AI*	47.5[a]	55.7[a]	28.3[b]
Overall	57.6[a]	55.7[a]	30.0[b]

[ab] Within a row, values with different superscripts are different ($P < 0.05$).
SEM = Standard error of the mean.
* Fixed-time AI was performed at 54 h after PGF treatment in the EP group and at 72h after PGF treatment in the GnRH and Control groups.

8.4.2 Experiment 2

Pregnancy rates were not significantly different among groups (EB/EB, 36.7%; EB/GnRH, 33.3%; GnRH/EB, 32.2%; and GnRH/GnRH, 41.9%).

8.5 Discussion

The diameter of the largest follicle at the time of PGF treatment was significantly larger in the Control group than in the other two groups. Although sequential examinations were not conducted to monitor follicle development, we infer that the increased size of dominant follicles at the time of PGF treatment in some of the cattle in the Control group was associated with the development of persistent follicles. In that regard, the diameter of the largest follicle was 15 to 16 mm in 7 of 18 cattle in the Control group, whereas there were no cattle with follicles greater than 13 or 14 mm in diameter in the EP and GnRH groups, respectively. In previous studies, a dominant ovarian follicle was shown to persist when MGA was fed in the absence of a functional CL (Custer et al., 1994; Kojima et al., 1995).

In Experiment 1, overall pregnancy rates and pregnancy rates to fixed-time AI were significantly lower in the Control group than in either the GnRH or EP groups. The significantly lower fertility in the Control group was probably due to the development of persistent follicles. It has been reported that persistent follicles ovulate (after MGA is withdrawn), but the oocyte is aged and fertility is reduced (Custer et al., 1994; Kojima et al., 1995). In field trials, when beef cows and heifers (n = 138) were

given MGA (0.5 mg/day) for 7 days with PGF on the last day, the conception rate was significantly lower in cattle that were on Day 14 to Day 20 of the estrous cycle at the time MGA treatment was initiated (36%) compared to those that were on days 0 to 13 (66%; Beal et al., 1988).

In the present study, including either GnRH or estradiol (and progesterone) at the start of MGA treatment significantly reduced dominant follicle size at PGF treatment and was associated with improved fertility, likely due to the reduced incidence of persistent follicles. In the Ovsynch regimen (Pursley et al., 1995), the first dose of GnRH was intended to cause ovulation or atresia of large antral follicles, with a new wave emerging approximately 2 days later (Twagiramungu et al., 1995; Pursley et al., 1995). In previous studies, treatment with E-17β (and progesterone) caused atresia of existing antral follicles with emergence of a new follicular wave approximately 4 days after treatment (Bó et al., 1995a). Progesterone (typically 50 or 100 mg) was given with the E-17β to prevent an estrogen-induced LH release in cattle without a functional CL (Bó et al., 1994). Although estradiol benzoate has a longer half-life than E-17β (Bó et al., 1996; Vynckier et al., 1990), either can be used for both synchronization of follicular wave emergence and synchronization of ovulation (Section 7.0). Furthermore, treatment with progesterone alone (200 mg) in MGA-treated cows induced atresia of persistent follicles, with relatively synchronous emergence of a new wave approximately 3 days later and improved fertility (Anderson and Day, 1994). Progesterone treatment has subsequently been incorporated into the middle of 20-day (Anderson and Day, 1998) or 14-day (McDowell et al., 1998) MGA-based synchronization regimens to obtain normal fertility at synchronized estrus.

Combined for all groups in Experiment 1, estrus rates averaged 59.8% for cows and heifers. However, these cattle were only observed twice-daily from 36 to 96 hours after PGF. Estrus rates can be highly variable, depending on the proportion of the cattle cycling prior to treatment, the nature of the treatment regimen, and the method of estrus detection (Odde, 1990). The principal purpose of detecting estrus is to determine the appropriate time to inseminate. However, treatments to synchronize the preovulatory gonadotropin surge have resulted in synchronous ovulation, facilitating fixed-time AI. Estrus detection and indeed whether or not estrus is expressed, is unimportant if ovulation is synchronized in all or most cattle. In the Ovsynch regimen, the second dose of GnRH was intended to induce ovulation in a majority of treated cattle (Pursley et al., 1995). In estradiol-based regimens, estradiol has been given after PGF treatment to increase the proportion of cattle in estrus and the synchrony of the estrus behaviour and ovulation (Dailey et al., 1983; 1986). When 0.5 mg (Hanlon et al., 1996) or 1.0 mg (Rasby et al., 1998) of EB was given 24 hours after CIDR-B removal, the proportion of heifers detected in estrus (96 and 81%, respectively) was higher than in untreated Control groups (90 and 37%, respectively). In another study, treatment with EB (0.38 or 1.0 mg in heifers and cows, respectively) 24 to 30 hours after CIDR-B removal in a 7-day treatment regime effectively induced estrus (estrus rate, 86 and 100% respectively; Lammoglia et al., 1998).

Although pregnancy rates to fixed-time AI were not significantly different between the GnRH and EP groups in both experiments, there are indications that estradiol may be preferable to GnRH. In Experiment 1, the interval from PGF treatment to estrus was shortest and least variable in the EP group, suggesting that a single fixed-

time insemination would be more successful in this group. In a previous study (Thundathil et al., 1999), beef cows were treated with 5 mg E-17β (and 100 mg progesterone) or 100 μg GnRH on the first day of a 7-day MGA regimen with PGF on the last day; synchronized conception rates were 47.5 and 57.5%, respectively in the first experiment (n = 141) but were 64.7 and 33.3 in a second experiment (n = 78). It was concluded that 5 mg E-17β (and progesterone) more reliably resulted in acceptable estrus and pregnancy rates than 100 ug GnRH. In a study designed to determine synchrony of follicular wave emergence, only 15 of 27 (55.6%) heifers ovulated in response to GnRH (100 μg gonadorelin) treatment administered at various times during the growth of the dominant follicle of the first follicular wave (Martínez et al., 1999). There was no indication that GnRH treatment induced follicle atresia nor did it alter the interval to wave emergence in the heifers that failed to ovulate. Overall, follicular wave emergence occurred from 2 days before to 7 days after GnRH treatment.

Although pregnancy rates were not significantly different among treatments in Experiment 2, they were disappointingly low. In retrospect, the change in diet may have adversely affected fertility. Unfortunately, an ultrasound examination was not done prior to the initiation of treatments. It has been reported that decreasing the feed intake from 1.2 x to 0.4 x maintenance suppressed ovulation in two of eight heifers, and in the remaining six heifers, the growth rate and maximum diameter of the first dominant follicle after ovulation were also reduced (Mackey et al., 2000). In another study, the number of small follicles on days 1 and 2 of the first follicular wave was 37% higher in cattle fed twice maintenance diets compared to those fed Control or feed-deprived diets (Gutierrez et al., 1997). In that study, a short-term increase in the level of nutrition

increased follicular recruitment. In contrast, serum concentrations of LH, estradiol and IGF-I decreased before the onset of nutritionally induced anovulation in beef heifers, reducing the growth rate and diameter of the ovulatory follicle (Bossis et al., 1999) and shortening the interval of persistence of the dominant follicle (Murphy et al., 1991). A reduction in dominant follicle diameter (e.g. < 9 mm) may decrease the proportion of follicles that ovulate in response to GnRH treatment (Martínez et al., 1999). Reduced LH pulse frequency would also inhibit CL development and function, potentially increasing the incidence of embryonic loss.

In conclusion, both GnRH and estradiol treatments designed to synchronize follicular wave emergence and ovulation resulted in acceptable pregnancy rates to fixed-time AI in an MGA-based synchronization regimen. However, the interval from PGF treatment to estrus was shortest and least variable in cattle treated with estradiol.

9.0 THE USE OF A PROGESTERONE-RELEASING DEVICE (CIDR-B) OR MELENGESTROL ACETATE WITH GnRH, LH OR ESTRADIOL BENZOATE FOR FIXED-TIME AI IN BEEF HEIFERS

9.1 Abstract

The objective of this experiment was to compare two progestins and three treatments for synchronizing follicular wave emergence and ovulation in protocols for fixed-time AI in beef heifers. On Day 0 (beginning of the experiment), Angus and Angus-Simmental cross beef heifers at random stages of the estrous cycle either received a CIDR-B device (n = 257) or were started on 0.5 mg/animal/d melengestrol acetate (MGA; n = 246) and were randomly assigned to receive i.m. injections of: 100 μg GnRH; 12.5 mg porcine LH (pLH); or 2 mg estradiol benzoate (EB) and 50 mg progesterone (P). The last feeding of MGA was given on Day 6, and on Day 7, CIDR-B devices were removed and all heifers received 500 μg cloprostenol (PGF). Consistent with their treatment groups on Day 0, heifers were given either 100 μg GnRH or 12.5 mg pLH 48 h after PGF (and were concurrently inseminated) or 1 mg EB 24 h after PGF and were inseminated 28 h later (52 h after PGF). Estrus rate (combined for both progestins) in heifers receiving EB (92.0%) was greater ($P < 0.05$) than for heifers receiving GnRH and pLH (combined) and a CIDR-B device (62.9%) or MGA (34.3%). Although the mean interval from PGF treatment to estrus did not differ among groups

(overall, 47.8 h; P = 0.85), it was less variable (P < 0.01) in MGA-fed heifers (SEM = 0.2 h) than in CIDR-B-treated heifers (SEM = 0.5 h). Pregnancy rates (determined by ultrasonography approximately 30 days after AI) did not differ (P = 0.30) among the six treatment groups (average, 58.0%; range 52.5 to 65.0%). Although fixed-time AI was done, pregnancy rates were greater in heifers detected in estrus than those not detected in estrus (62.6 versus 51.9%; P < 0.05). In conclusion, GnRH, pLH, or EB treatment in combination with a CIDR-B device or MGA effectively synchronized ovulation for fixed-time AI, resulting in acceptable pregnancy rates in beef heifers.

9.2 Introduction

Current approaches to fixed-time AI in cattle involve a source of progestin, and synchronization of follicular wave emergence, the preovulatory LH surge, and ovulation (De Rensis and Peters, 1999). One protocol (Ovsynch) consists of two injections of GnRH (8 or 9 days apart), PGF 30 to 48 h before the second GnRH, and timed AI, 0 to 24 h after the second GnRH (Risco et al., 1998). The first GnRH is to synchronize follicular wave emergence and the second is to induce the preovulatory LH surge and ovulation. However, this protocol has not resulted in acceptable pregnancy rates in heifers (Pursley et al., 1997; Risco et al., 1998). Failure of ovulation to the first GnRH (Martínez et al., 1999) and asynchronous onset of estrus relative to the second GnRH may account for the reduced fertility in some heifers (Risco et al., 1998). The insertion of a progesterone-releasing intravaginal device (CIDR-B) or feeding melengestrol acetate (MGA) provides the basis for many fixed-time AI programs. The

addition of a CIDR-B to an Ovsynch program improved pregnancy rates in heifers (68.0 versus 39.1%; Section 10.0). Porcine LH (pLH) has also been used in lieu of GnRH in an Ovsynch-type program (Section 10.0). Estrogens have also been shown to effectively synchronize follicle wave emergence (Bó et al., 1995a, 1996; Caccia and Bó, 1998) and a preovulatory LH surge (Lammoglia et al., 1998) and have been used in synchronization programs with a CIDR-B (Martínez et al., 2000) or MGA (Kastelic et al., 1997). Estradiol resulted in a greater pregnancy rate than GnRH in a CIDR-B-based fixed-time AI program (Section 7.0), with no difference when MGA was used (Section 8.0).

The objective of the present study was to compare the two progestins (CIDR-B and MGA) and three treatments that we have used previously to synchronize follicular wave emergence and ovulation (GnRH, pLH and estradiol benzoate) in a fixed-time AI program in beef heifers.

9.3 Materials and Methods

Angus (Black and Red) and Angus-Simmental cross heifers, approximately 15 mo of age and weighing from 275 to 350 kg (body condition score approximately 3.25) were used in this study. Heifers had been in a feedlot for approximately 7 mo and were fed approximately 5 kg barley silage and 1 kg of rolled barley/animal/d. Heifers were housed outdoors in feedlot pens (approximately 50 or 100 heifers per pen, 50 m^2/animal).

Transrectal ultrasound examinations were conducted to determine the presence of a corpus luteum (CL) in order to confirm cyclicity, and that heifers were nonpregnant and free of morphologic abnormalities of the reproductive tract. Heifers were randomly assigned to one of six groups in a 2 x 3 factorial design (Figure 1). On Days 0, 257 heifers had a CIDR-B device containing 1.9 g of progesterone (Vetrepharm Canada Inc, Belleville, ON, Canada) placed in the vagina. The tails of the CIDR-B devices were cut even with the vulva to prevent pen mates from pulling them out. The remaining heifers (n = 246) were given 0.5 mg/animal/d MGA in their feed (Pharmacia Animal Health, Orangeville, ON, Canada) from Day 0 to Day 6. On Day 0, heifers were given i.m. injections of: 100 μg GnRH (Cystorelin; Merial Canada Inc, Victoriaville, PQ, Canada), 12.5 mg pLH (Lutropin-V; Vetrepharm Canada Inc), or 2 mg estradiol benzoate (EB; Sigma-Aldrich Can Ltd, Oakville, ON, Canada) and 50 mg progesterone (Sigma-Aldrich) in 2 mL canola oil. On Day 7, CIDR-B devices were removed and all heifers received an i.m. injection of 500 μg cloprostenol (PGF; Estrumate, Schering-Plough Animal Health, Pointe-Claire, PQ, Canada). Consistent with their treatment groups on Day 0 (GnRH, pLH or estradiol), heifers were given either 100 μg GnRH or 12.5 mg pLH 48 h after PGF and concurrently inseminated or 1 mg EB in 2 mL canola oil i.m. (Sigma-Aldrich) 24 h after PGF and inseminated, approximately 28 h later 52 h after PGF; the LH peak is expected to occur approximately 18 h after treatment with estradiol; Bó et al., 1994)]. Frozen-thawed semen from a single sire was used and the same operator performed all inseminations. Although observations for behavioural estrus were conducted at least twice daily from

24 to 72 h after PGF treatment to determine estrus synchrony, all heifers were fixed-time inseminated.

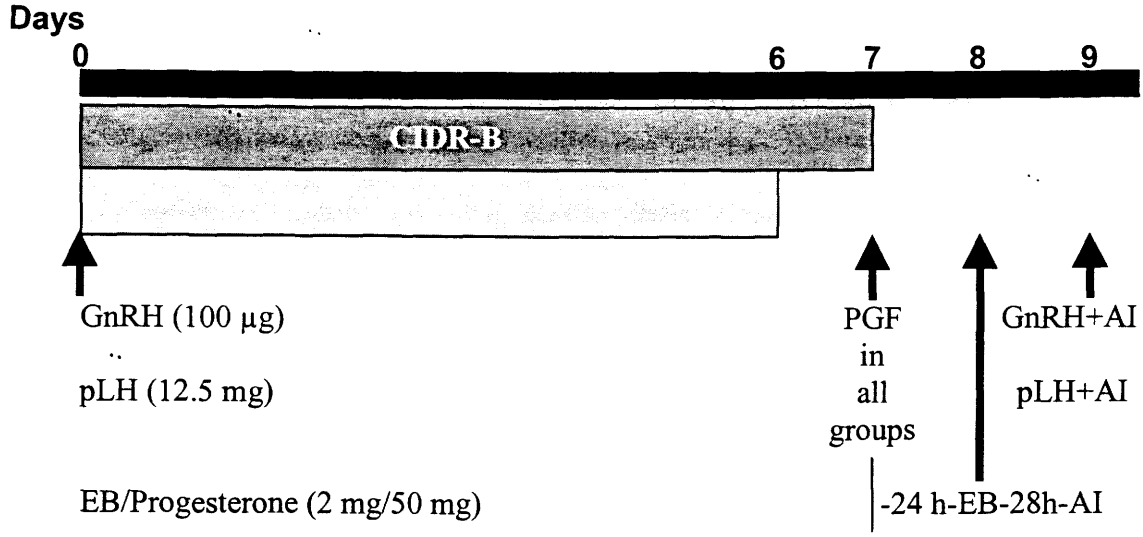


Figure 9.1 Schematic outline of the experiment (arrows indicate times of administration of treatments). The CIDR-B devices were removed after 7 days and MGA was fed once daily from Days 0 to 6. Heifers were treated with GnRH, pLH or estradiol benzoate (EB) and progesterone on Day 0 and all received PGF on Day 7. Heifers that received GnRH or pLH received a second injection of the same hormone 48 h after PGF (Day 9) and were inseminated concurrently, while those in the EB group received EB 24 h after PGF (Day 8) and were inseminated 28 h later (52 h after PGF; Day 9).

Heifers were retained in the feedlot and exposed to fertile bulls for 17 d, starting approximately 20 days after AI. Pregnancy was diagnosed by transrectal ultrasound examination approximately 30 days after AI (Curran et al., 1986). Following bull exposure, heifers were sent to pasture. Fall pregnancy rate was determined by rectal palpation, conducted approximately 150 days after fixed-time AI. A heifer diagnosed as

pregnant at the first examination but subsequently diagnosed non-pregnant in the fall was defined as having a pregnancy loss.

Mean and standard error of the mean (SEM) were used to describe data. A two-way analysis of variance was used to determine the effect of progestin, synchronizing treatment, and their interaction, on the interval from PGF treatment to estrus. Bartlett's test was used to compare the variances in the interval from PGF treatment to estrus among the six treatment groups, between the two progestins, and among the three synchronizing treatments. Logistic regression analysis was used to compare the effect of progestin, treatment, and progestin-by-treatment interaction on estrus and pregnancy rates. Embryonic loss rate was compared among the six treatment groups, between the two progestins, and among the three synchronizing treatments by Chi-square analysis. Chi-square was also used to compare the pregnancy rate between heifers that showed estrus and those that did not. All statistical analyses, except logistic regression (SPSS, version 10.05, 1999, SPSS Inc., Chicago, IL, USA), were conducted using SAS (SAS Institute Inc., Cary, NC, USA).

The protocols for these experiments were approved by University of Saskatchewan Animal Care Committee.

9.4 Results

The distribution of estrus is shown in Table 9.1 and Figure 9.2. Reproductive performance according to presence or absence of detected estrus is shown in Table 1. The proportion of heifers detected in estrus was greatest in EB groups (combined for

both progestins, 92.0%) and least in GnRH or pLH groups fed MGA (35.6 and 33.0%, respectively). Estrus was detected in a greater ($P < 0.0001$) proportion of heifers given a CIDR-B device than in those fed MGA (68.9 versus 45.5%). The mean interval from PGF treatment to estrus was not different among groups (overall, 47.8 h; $P = 0.85$) and the modal interval from PGF to estrus was 48 h. However, the interval from PGF treatment to estrus was less variable ($P < 0.01$) in heifers fed MGA (SEM = 0.2 h) than in those treated with a CIDR-B (SEM = 0.5 h). There was an effect of progestin ($P < 0.001$), but there was no effect of treatment ($P = 0.72$), or progestin-by-treatment interaction ($P = 0.11$) on estrus rate (overall mean, 58.0%). There was no effect of progestin ($P = 0.11$), treatment ($P = 0.26$), or progestin-by-treatment interaction ($P = 0.22$) on pregnancy rate to fixed-time AI (overall mean, 58.0%). Combined for all treatments, pregnancy rate was greater ($P < 0.05$) in heifers detected in estrus (62.6%) than in those not detected in estrus (51.9%). Fall pregnancy rate was not different ($P = 0.86$) among groups (overall mean, 77.1%). Pregnancy losses were not different among groups and ranged from 5.3% in CIDR-B-treated heifers that were synchronized with pLH to 13.0% in MGA-treated heifers synchronized with pLH (overall mean, 9.3%).

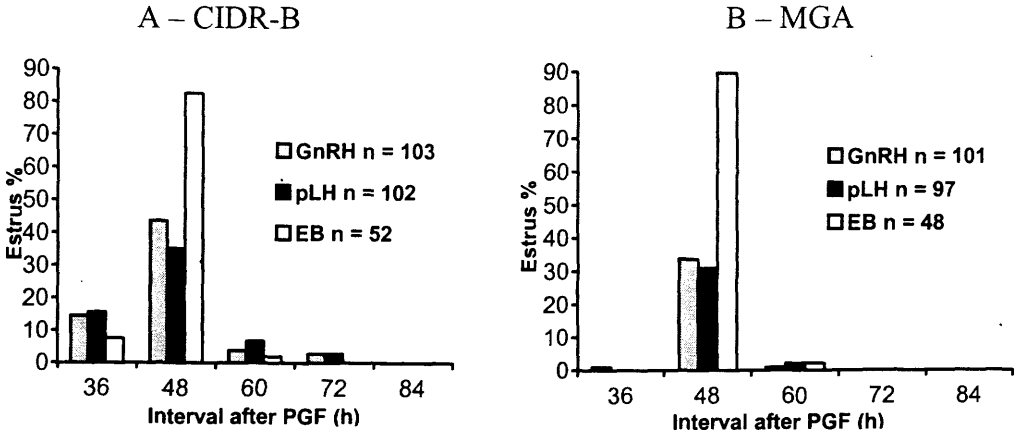


Figure 9.2 Distribution of estrus after PGF administration in heifers that received a CIDR-B device (A) or were fed MGA (B) and treated with GnRH, pLH or estradiol benzoate (EB) to synchronize follicular wave emergence and ovulation for fixed-time AI.

Table 9.1 Estrus rates, interval from PGF to estrus, and pregnancy rates in beef heifers receiving a CIDR-B device or fed MGA and given GnRH, pLH or EB for the synchronization of follicular wave emergence and ovulation for fixed-time AI[1]

	CIDR-B			MGA		
	GnRH	pLH	EB	GnRH	pLH	EB
No. heifers	103	102	52	101	97	48
Estrus %	65.0^{a}	60.8^{a}	92.3^{b}	35.6^{c}	33.0^{c}	91.7^{b}
PGF to estrus h						
Mean	47.1	47.8	47.3	48.0	48.8	48.3
SEM[2]	0.8^{x}	1.0^{x}	0.5^{y}	0.3^{y}	0.3^{y}	0.3^{z}
Pregnancy rate to AI %						
Overall	65.0	55.9	61.5	52.5	55.7	60.4
Detected in estrus	67.2	61.3	62.5	61.1	62.5	59.1
Not detected in estrus	61.1	47.5	50.0	47.7	52.3	75.0
Fall pregnancy rate %	75.7	77.4	78.8	73.3	72.2	77.1

^{abc} Mean and percentages without a common superscript differ ($P < 0.05$)
^{xyz} Variances without a common superscript differ ($P < 0.05$)
[1] Please refer to Figure 1 for treatment schedule; PGF was administered to all heifers on Day 7 at CIDR-B removal (1 day after the end of MGA feeding)
[2] Standard error of the mean was calculated for simple effects to show differences in variation of the interval from PGF to estrus but statistical comparisons were done with variances.

9.5 Discussion

The use of progestins in estrus synchronize programs has become widespread in the last 10 yr (De Rensis and Peters, 1999). In recent reports (Sections 7.0, 8.0), pregnancy rates to fixed-time AI were greater in cattle that received a CIDR-B than in

those fed MGA. As pregnancy rates to fixed-time AI in the present study were not significantly different among groups (overall mean, 58.0%), factors other than pregnancy rate (e.g., costs and management) may determine the program selected. For example, CIDR-B devices are more expensive than MGA, but they can be used in both confined cattle and those at pasture.

Although MGA is marketed for the suppression of estrus in feedlot heifers, it has also been used in estrus synchronization programs (Patterson et al. 1989; Odde 1990). Feeding of MGA for 14 to 17 days effectively synchronizes estrus, but results in low fertility (Odde, 1990). However, feeding MGA for shorter periods of time (e.g., 7 d) has resulted in improved fertility when feeding was started early in the estrous cycle, but not when MGA was started late in the cycle (Beal et al. 1988); this is probably due to the development of persistent dominant follicles (Custer et al. 1994; Kojima et al. 1995) which are associated with reduced fertility (Patterson et al., 1989; Custer et al., 1994; Ahmad et al., 1995). This problem may be overcome by strategically synchronizing follicular wave emergence at the start of MGA treatment.

It has been shown that estradiol synchronizes emergence of a new follicular wave by suppressing FSH (Bó et al., 1995a). Estradiol and progesterone given at the beginning of a CIDR-B protocol in beef heifers was followed by follicle regression and the synchronous emergence of a new follicular wave, 3.4 ± 0.1 days later (Martínez et al., 2000). Therefore, in a 7-day protocol (as used in the present study), the dominant follicle was expected to be on approximately days 3 or 4 of its growing phase (9 to 10 mm) at the time of CIDR-B removal or discontinuation of MGA.

There has been continuing development of CIDR-B-based programs in the last several years. Initially, EB was delivered by a gelatin capsule placed in the vagina along with the CIDR-B device (Macmillan et al., 1991). Subsequent work showed that the i.m. administration of EB resulted in more consistent and synchronous wave emergence (Bó et al., 1996). Estradiol-17β and progesterone have been incorporated into a 7-day MGA feeding program, resulting in acceptable fertility after estrus detection and AI (Kastelic et al., 1996). Estrogens and GnRH have been compared in CIDR-B based programs for synchronization of wave emergence and ovulation (Section 7.0). In one study, estrus and pregnancy rates to fixed-time AI were 100 and 76%, respectively, in CIDR-B-treated heifers given EB, compared with 55 and 48% in heifers given GnRH (Section 7.0). In another study, beef cows were given MGA for 7 days (Section 8.0); estradiol or GnRH were given to synchronize follicular wave emergence and ovulation, resulting in pregnancy rates to fixed-time AI of 55.7 and 47.5%, respectively. The results of the present study suggest that there is likely to be no difference in pregnancy rates to fixed-time AI between the use of CIDR-B or MGA and one of the three methods of synchronizing follicular wave emergence and ovulation.

We have previously shown that the administration of estradiol when circulating levels of progesterone are low will induce LH release, incomplete suppression of the dominant follicle, and a delay in the emergence of the next follicular wave (Bó et al., 1994). Therefore, we included progesterone with the first estradiol treatment in the present study. However, the inclusion of progesterone with EB at the time of CIDR-B insertion has been reported to have no effect on pregnancy rate (Bó et al., 2000b). Plasma progesterone concentrations have been reported to increase by 2 h after CIDR-

B insertion (Burke et al., 1999) and may prevent an estrogen-induced LH surge in cattle without a functional CL. However, progestin levels may increase more slowly and be less suppressive with an oral preparation such as MGA (Kojima et al., 1995). In addition, progesterone along with estradiol may improve its efficacy in inducing regression of larger antral follicles (Anderson and Day, 1994, 1998; McDowell et al., 1998).

Estrogen treatment has also been given after PGF administration to increase the proportion of cattle in estrus and the synchrony of estrus behaviour (Dailey et al., 1983; 1986). Bó et al. (1994) reported that an LH surge occurred 16 to 18 h after an injection of estradiol in animals without a functional CL. The administration of EB following CIDR-B removal has been shown to result in a greater estrus rate than that in Control heifers (Hanlon et al., 1996) and an LH surge, and ovulation (Lammoglia et al., 1998). In the present study, EB appears to have been very effective in synchronizing both the emergence of an ovulatory wave and ovulation of the dominant follicle of that wave.

Treatment with GnRH induces ovulation of large antral follicles, with a new follicular wave emerging approximately 2 days later (Twagiramungu et al. 1994, 1995). However, synchronous emergence of a new follicular wave occurs only when treatment causes ovulation. In an experiment in which GnRH or pLH was administered at various phases of the first follicular wave, ovulation of the dominant follicle was induced in 56 or 78% of heifers, respectively (Martínez et al., 1999). Heifers that failed to ovulate following the second GnRH treatment in an Ovsynch protocol were reported to be in metestrus or early diestrus at the first GnRH treatment (Pursley et al., 1995). Therefore, ovulation following PGF treatment may be poorly synchronized if the first GnRH

treatment does not induce ovulation of the dominant follicle and thereby fails to synchronize wave emergence.

The Ovsynch protocol has yielded acceptable pregnancy rates in both dairy (Pursley et al., 1997) and beef (Geary et al., 2001) cows, but pregnancy rates in heifers have been unacceptably low (Pursley et al., 1997). The addition of a CIDR-B device to a GnRH- or pLH-based Ovsynch program resulted in improved pregnancy rates in beef heifers (68 versus 39% or 65 versus 38%, respectively; Section 10.0). Therefore, progestins (CIDR-B or MGA) were used in the period between the first GnRH or pLH treatment and the administration of PGF in the present study; pregnancy rates to fixed-time AI were highly acceptable.

In the present study, the use of EB for synchronization of estrus and ovulation required one additional handling compared with GnRH or pLH treatment groups, but there was no difference in pregnancy rates. However, insemination 8 to 24 h after GnRH treatment has been reported to result in numerically the highest pregnancy rates (Pursley et al., 1998). Insemination a few hours after GnRH or pLH treatment may have improved pregnancy rates in the present study, but it would have required an additional handling. Although stress may affect pregnancy rates, estradiol treatment with an additional handling resulted in pregnancy rates that were at least as high as that of groups requiring less handling.

Heifers were fixed-time inseminated in the present study, but they were also observed for estrus. Although there was no effect of treatment group, estrus rate was numerically greater in EB-treated heifers (92.0%) than in those given GnRH (50.7%) or

pLH (47.2%) and was greater in heifers with a CIDR-B device (68.9%) than in those fed MGA (45.5%). High estrus rates have been previously reported for estrogen-treated cattle (Hanlon et al., 1996; Ryan et al., 1996; Day et al., 2000), but much lower in cattle given GnRH in an Ovsynch program (Stevenson et al., 1996). Obviously, many factors influence the expression and detection of estrus (Loeffler et al., 1999), and in the present experiment, the use of a CIDR-B device significantly increased the expression of estrus. However, synchronization of follicle wave emergence and the preovulatory LH surge (and ovulation) in fixed-time AI programs preclude the need for estrus detection. Nevertheless, pregnancy rates were greater in GnRH- or pLH treated heifers that were detected in estrus, but pregnancy rates were not different in EB-treated heifers.

Because pregnancy rate is the product of estrus rate and conception rate, pregnancy rate will be low if either is low, or if both are modest. Fixed-time AI eliminates variability associated with estrus detection and it may be optimized if ovulation is synchronous; this can be facilitated by synchronizing wave emergence and the preovulatory LH surge. The programs used in the present experiment were successful in achieving this objective.

Implications

The use of GnRH, porcine LH or estradiol benzoate in combination with an intravaginal progesterone-impregnated device or feeding melengestrol acetate apparently synchronized ovulation (and follicular wave emergence), facilitating fixed-time AI in beef heifers. Pregnancy rates to a single fixed-time insemination were highly acceptable in all groups (overall average, 58%). Factors including product cost and availability, animal management, and handling will influence the decision of which program will be best suited to a particular beef operation.

10.0 THE USE OF PROGESTINS IN REGIMENS FOR FIXED-TIME ARTIFICIAL INSEMINATION IN BEEF CATTLE

10.1 Abstract

Four experiments were conducted to investigate modifications to GnRH-based fixed-time AI protocols in beef cattle. In Experiment 1, the effect of reducing the interval from GnRH treatment to PGF was examined. Lactating beef cows (n = 111) were given 100 μg gonadorelin (GnRH) on Day 0 (start of treatment) and either 500 μg cloprostenol (PGF) on Day 6 with AI and 100 μg GnRH 60 h later, or PGF on Day 7 with AI and GnRH 48 h later (6- or 7-day Cosynch regimens). Pregnancy rates were 32/61 (52.4%) versus 26/50 (52.0%), respectively (P = 0.96). In Experiment 2, cattle (n = 196) were synchronized with a 7-day Cosynch regimen and received either no further treatment or a CIDR-B device (Days 0 to 7). Pregnancy rates were 32/71 (45.1%) versus 33/77 (42.9%) in cows (P < 0.8), and 9/23 (39.1%) versus 17/25 (68.0%) in heifers (P < 0.05). In Experiment 3, 49 beef heifers were randomly assigned to receive 12.5 mg pLH on Day 0, PGF on Day 7 and 12.5 mg of pLH on Day 9 with AI 12 h later (pLH Ovsynch), or similar treatment plus a CIDR-B device from Days 0 to 7 (pLH Ovsynch+CIDR-B), or 1 mg estradiol benzoate (EB) and 100 mg progesterone on Day 0, a CIDR-B device from Days 0 to 7 (EB/P4+CIDR-B), PGF on Day 7 (at the time of CIDR-B removal) and 1 mg im EB on Day 8 with AI on Day 9 (52 h after PGF). Pregnancy rate in the EB/P4+CIDR-B group (75.0%) was higher (P < 0.04) than in the

pLH Ovsynch group (37.5%); the pLH Ovsynch+CIDR-B group was intermediate (64.7%). In Experiment 4, 266 non-lactating cows were allocated to a 7-day Cosynch protocol (Cosynch), a 7-day Cosynch plus 0.6 mg/head/d melengestrol acetate (MGA) from Days 0 to 6 inclusive (Cosynch+MGA) or MGA (Days 0 to 6) plus 2 mg EB and 50 mg progesterone on Day 0, 500 μg PGF on Day 7, 1 mg EB on Day 8 and fixed-time AI 28 h later (EB/P+MGA). Pregnancy rates ($P < 0.25$) were 44.8% (39/87; Cosynch), 47.8% (43/90; Cosynch+MGA), and 60.7% (54/89; EB/P+MGA). In conclusion, a 6- or 7-day interval from GnRH to PGF in a Cosynch regimen resulted in similar pregnancy rates in cows. The addition of a progestin to a Cosynch or Ovsynch regimen significantly improved pregnancy rates in heifers but not in cows. Progestin-based regimens that included EB consistently resulted in high pregnancy rates to fixed-time AI.

10.2 Introduction

Gonadotropin releasing hormone (GnRH) has been used in many fixed-time AI programs (Adams, 1998; Pursley et al., 1995; Twagiramungu et al., 1995; Wiltbank, 1997). Usually, GnRH is given twice in a 9- to 10-d period. When GnRH is given at the start of a synchronization regimen, it is expected to induce ovulation of the extant dominant follicle, followed by the emergence of a new follicular wave approximately 2 days later (Pursley et al., 1995; Twagiramungu et al., 1995; Martínez et al., 2000). Prostaglandin (PGF) treatment is given 6 or 7 days later to induce regression of luteal

tissue (Pursley et al., 1995; Twagiramungu et al., 1995). In the "Ovsynch" regimen, a second dose of GnRH is given after PGF treatment to synchronize the LH surge and ovulation, enabling fixed-time AI 0 to 24 h later (Pursley et al., 1995). This protocol has resulted in acceptable pregnancy rates (approximately 50%) in beef cows (Geary et al., 2001; Thundathil et al., 1999). Furthermore, the administration of the second dose of GnRH, concurrently with fixed-time AI ("Cosynch") in beef cows, resulted in a pregnancy rate equal to that of a group inseminated 24 h later (Geary et al., 2001). The Ovsynch regimen has not been widely used in heifers, since pregnancy rates have been low (Wiltbank, 1997).

Cattle displaying estrus prior to PGF treatment in an Ovsynch regimen are unlikely to become pregnant after fixed-time AI. The incidence of early estrus was 6.4 and 8.9% in 6-day regimens in cows (Roy and Twagiramungu et al., 1996), 12.5 and 11.8% in 7-day regimens in cows (Roy and Twagiramungu et al., 1999; Seguin, 1997), and 17.3% in a 6-day regimen in heifers (Roy and Twagiramungu et al., 1996). The higher incidence of premature estrus (and poor fertility) in heifers may be the result of inconsistent response to the first GnRH treatment; ovulation occurred in only 50% (Martínez et al., 2000) and 56% (Pursley et al., 1995) of GnRH-treated heifers. In cows, however, ovulation rate after the first GnRH ranged from 64% (Vasconcelos et al., 1999) to 90% (Pursley et al., 1995). Induction of ovulation of the dominant follicle is important as it is followed by the emergence of a new follicular wave; it was concluded that the timing of follicular wave emergence was not affected when ovulation did not occur after GnRH treatment (Martínez et al., 1999). It has been reported that shortening

the interval from 7 days to 6 days results in a reduced incidence of premature estrus and greater precision of estrus (Roy and Twagiramungu, 1996, 1999). We hypothesized that providing an exogenous source of progestin during the interval from the first GnRH to PGF treatment would reduce the incidence of premature estrus. Alternatively, estradiol can be used in lieu of GnRH for synchronization of wave emergence and ovulation in progestin-treated cattle (Martínez et al., 1999). Estradiol treatment of progestin-treated cattle results in a new follicular wave 4 or 5 days later, while estradiol treatment of animals with low levels of progesterone results in a LH surge 18 to 24 h later (Bó et al., 1994) and ovulation approximately 30 h after the LH surge (Kojima et al., 1995).

Four experiments were conducted to evaluate modifications to GnRH-based fixed-time AI protocols in beef cattle. The objective of Experiment 1 was to determine the effect of reducing the interval between the first injection of GnRH and PGF administration in lactating beef cows. Experiment 2 was designed to determine the benefit of including a CIDR-B device in a GnRH-based regimen in both cows and heifers. The objective of Experiment 3 was to compare two synchronization treatments involving a pLH-based Ovsynch protocol, with or without CIDR-B, and a CIDR-B-based protocol with estradiol benzoate (EB) in heifers. The objective of Experiment 4 was to compare two synchronization treatments involving a GnRH-based Cosynch protocol, with or without melengestrol acetate (MGA), and an MGA-based protocol with EB in cows.

10.3 Materials and Methods

10.3.1 Experiment 1

Lactating beef cows (n = 111, 45 to 70 days postpartum) were body condition scored from 1 to 5 (Domecq et al., 1995), examined with transrectal ultrasonography (Aloka SSD-500 with a 7.5 MHz linear-array transducer; Instruments for Science and Medicine, Vancouver, BC, Canada) to determine the presence and size of the corpus luteum (CL), and randomly assigned to either a 6-day or 7-day Cosynch regimen (Figure 1). For the 6-day regimen, cows were given 100 μg im gonadorelin (GnRH, Cystorelin™, Merial Canada Inc, Victoriaville, PQ, Canada) on Day 0 (start of the experiment), followed by 500 μg cloprostenol (PGF, Estrumate™, Schering-Plough Animal Health, Pointe-Claire, PQ, Canada) on Day 6. Cows were artificially inseminated (AI) on Day 8.5 (60 h after PGF) and concurrently given 100 μg GnRH. For the 7-day regimen, cows received GnRH on Day 0, PGF on Day 7, and AI concurrently with GnRH on Day 9, (approximately 48 h after PGF). All hormone treatments were administered by im injection. The interval from PGF to GnRH and AI was approximately 12 h longer in the 6-day versus the 7-day regimen so that the diameter of the preovulatory follicle at the time of the second GnRH treatment would be approximately the same in both groups. Cows were observed for signs of estrus at 48 and 60 h after PGF treatment; inseminations using semen from 3 different bulls were done by a single inseminator. Pregnancy was determined by transrectal ultrasonography approximately 30 days after AI. Estrus and pregnancy rates were compared by Chi-

square analysis (Norman and Streiner, 2000; Statistix for Windows™, Analytical Software, Version 2.0, 1996. Tallahassee, FL, USA).

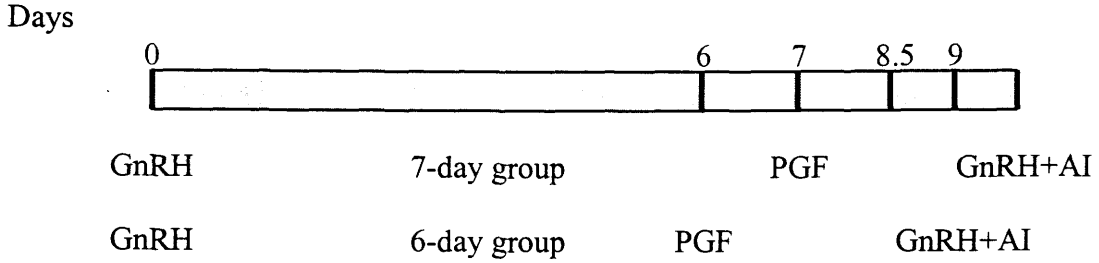


Figure 10.1 Treatment schedule for 7-day and 6-day Cosynch treatments. Cows were treated with GnRH and 6 (6-day) or 7 (7-day) days later with PGF. Cows in the 7-day group received a second injection of GnRH 48 h after PGF and were inseminated while those in the 6-day group received a second injection of GnRH 60 h after PGF and were inseminated.

10.3.2 Experiment 2

Lactating Simmental-cross cows (n = 148; 35 to 60 days postpartum) and heifers (n = 48) were treated in a 7-day program (as in Experiment 1). Animals were body condition scored and examined with transrectal ultrasonography to determine the presence and size of CL. They were randomly assigned to receive either no further treatment or a once-used CIDR-B device (Vetrepharm) inserted at the time of the first GnRH treatment (Day 0) and treatment with 500 μg im PGF at CIDR-B removal (Day 7; Figure 9.2). Forty-eight hours later, heifers were inseminated by a single inseminator and received a second injection of GnRH. Transrectal ultrasonography was conducted

24 days after AI to determine pregnancy status, and pregnancy rates for cows and heifers were compared separately by Chi-square analysis.

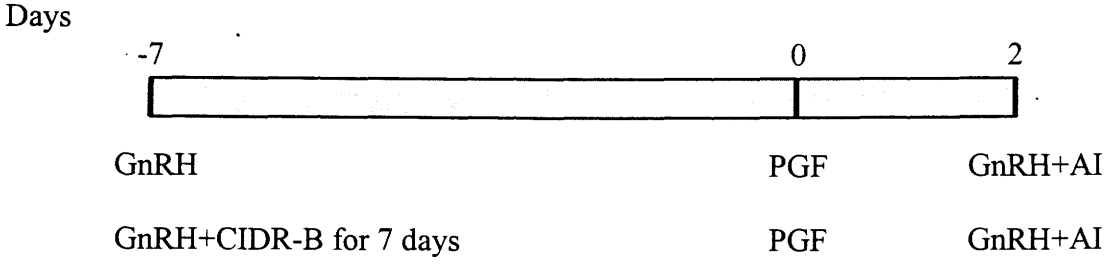


Figure 10.2 Treatment schedule for Ovsynch and Ovsynch+CIDR-B treatment groups. Cattle were treated with GnRH and 7 days later with PGF. Forty-eight hours later, they were inseminated and received a second injection of GnRH. Cattle in the first group received no further treatment and those in the second group also received a CIDR-B device from the time of the first injection of GnRH until the injection of PGF.

10.3.3 Experiment 3

Forty-nine beef heifers were randomly assigned to three treatment groups (Figure 3). Heifers in the first group (pLH Ovsynch) received 12.5 mg pLH (Lutropin-V™, Vetrepharm) on Day 0, 500 μg PGF on Day 7 and 12.5 mg of pLH on Day 9 with AI 12 h later. Heifers in the second group (pLH Ovsynch+CIDR-B) were treated similarly, with the addition of a new CIDR-B device from Days 0 to 7. Heifers in the third group (EB+CIDR-B) were given 1 mg EB (Sigma Chemical Co, St. Louis, MO, USA) and 100 mg progesterone (Sigma) in canola oil im on Day 0, and a new CIDR-B device from

Days 0 to 7. Heifers were given PGF on Day 7 (at the time of CIDR-B removal) and 1 mg EB im on Day 8 with AI to a single bull done by a single inseminator on Day 9 (52 h after PGF). Heifers in all 3 groups were observed for estrus every 12 h from 24 to 60 h after PGF. Transrectal ultrasonography was conducted 30 days after AI to determine pregnancy status. The interval from PGF treatment to estrus was analyzed by Kruskal-Wallis nonparametric one-way analysis of variance and variances were compared with a Bartlett's test. Pregnancy rates were compared by Chi-square analysis (Norman and Streiner, 2000).

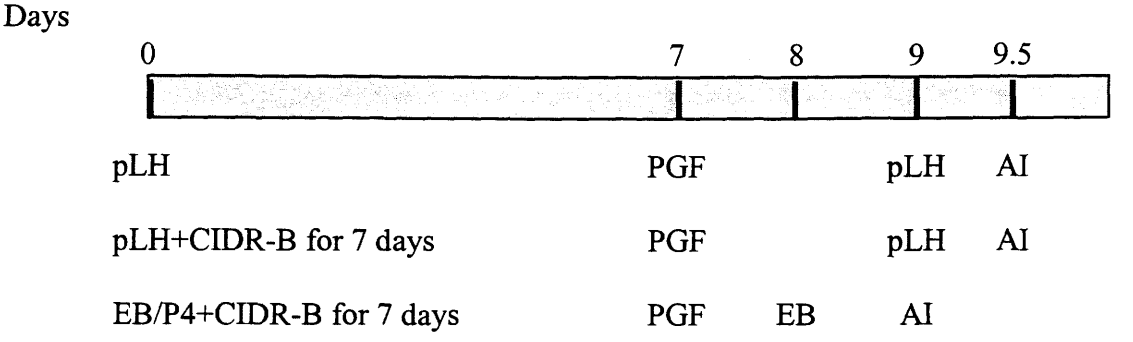


Figure 10.3 Treatment schedule for pLH-based Ovsynch regimens with or without a CIDR-B device for 7 days. Heifers in the first 2 groups received an injection of pLH on Day 0, an injection of PGF on Day 7, a second injection of pLH on Day 9 and AI 12 h later; heifers in the second group also received a CIDR-B device between Days 0 and 7. Heifers in the third group received an injection of estradiol benzoate and progesterone (EB/P4) along with a CIDR-B device on Day 0, an injection of PGF at the time of CIDR-B removal on Day 7, a second injection of EB 24 later (Day 8) and AI approximately 28 h later.

10.3.4 Experiment 4

Non-lactating, culled crossbred beef cows (n = 266) of unknown reproductive history were used. The cows were housed in group pens at a feedlot and were fed a diet of silage and screening pellets. Cows were examined by transrectal ultrasonography and those that were pregnant or had detectable abnormalities were excluded. Remaining cows (n = 249) were randomly allocated to three treatment groups (Figure 4). Cows in the first group (Cosynch) received 100 μg im GnRH on Day 0, 500 μg of PGF on Day 7, and 100 μg GnRH im concurrent with AI by a single inseminator using semen from a single bull 50 h after PGF. To maximize fertility in cows showing premature estrus, all cows in this group were observed twice-daily from Days 3 to 8 for standing estrus, and those that were detected in estrus were inseminated approximately 12 h later by the same operator and were not given the previously scheduled injections. These animals were considered to be not pregnant to the fixed-time AI. Cows in the second group (Cosynch+MGA) were treated similarly, except that they were also fed MGA (0.6 mg head/d; Pharmacia and Upjohn, Orangeville, ON, Canada) in a grain mix from Days 0 to 6 inclusive and given 100 μg im GnRH concurrent with AI approximately 50 h after PGF. Cows in the third group (EB/P+MGA) were also fed MGA from Days 0 to 6, but received an im injection of 2 mg EB and 50 mg progesterone on Day 0, 500 μg im PGF on Day 7, 1 mg im EB on Day 8 (approximately 24 h after PGF), and were inseminated by the same operator approximately 28 h later (52 h after PGF treatment). Semen from a single sire was used for all inseminations. Transrectal ultrasonography was conducted

approximately 30 days after AI to determine pregnancy status, and pregnancy rates were compared by Chi-square analysis.

The protocols for these experiments were approved by University of Saskatchewan Animal Care Committee.

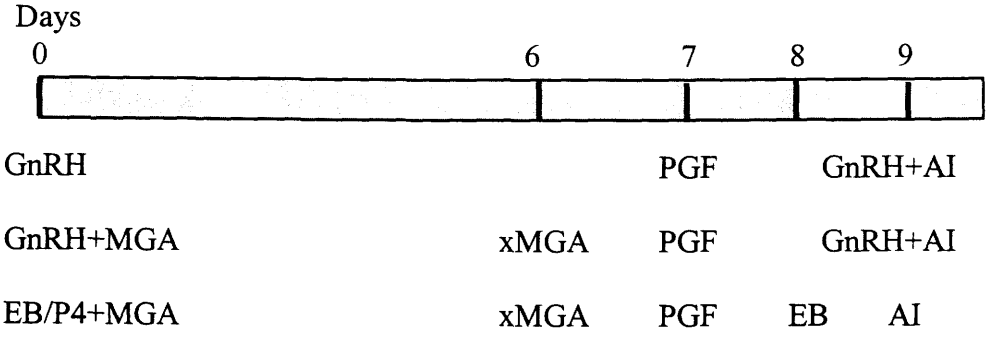


Figure 10. 4 Treatment schedule for GnRH-based Cosynch regimens with or without feeding MGA. Cows in the first 2 groups received an injection of GnRH on Day 0, an injection of PGF on Day 7, a second injection of GnRH on Day 9 concurrently with AI; cows in the second group were also fed MGA between Days 0 and 6. Cows in the third group received an injection of estradiol benzoate and progesterone (EB/P4) and were fed MGA from Days 0 to 6, an injection of PGF on Day 7, a second injection of EB 24 h later (Day 8) and AI approximately 28 h after EB.

10.4 Results

10.4.1 Experiment 1

All cows had an ultrasonically detectable CL (range in diameter, 8 to 27 mm) on Day 0. Estrus rates were 24.6 and 26.0% (P = 0.56), and pregnancy rates were 52.4%

(32/61) and 52.0% (26/50; P = 0.96) for the 6-day and 7-day Cosynch protocols, respectively (Table 10.1). There were 22, 72 and 17 cows with BCS of 2.0, 2.0 to 3.0, and 3.0, respectively. For these three categories, pregnancy rates were 31.8, 54.2 and 70.6%, respectively ($P < 0.05$; 31.8 versus 70.6%, $P < 0.02$). There was no bull effect on pregnancy rates ($P = 0.70$).

10.4.2 Experiment 2

Results are shown in Table 10.1. Pregnancy rates were 45.1% (32/71) for Cosynch cows (without a CIDR-B) and 42.9% (33/77) for Cosynch+CIDR-B cows (with a CIDR-B; $P = 0.79$). For the corresponding treatments in heifers, pregnancy rates were 39.1% (9/23) and 68.0% (17/25; $P < 0.05$). Fourteen heifers had a CL at the time of initiating treatments and, of these, the pregnancy rate was 4/7 and 5/7 for heifers without and with a CIDR-B, respectively. There were 53, 54 and 41 cows with a BCS 2.0, 2.0 to 3.0, and 3.0, respectively; pregnancy rates were 47.2, 46.3 and 36.6%, respectively, for these three categories ($P = 0.53$). It was not possible to rule out a bull effect because of the high number of bulls used (18 bulls in 148 cows and 20 bulls in 48 heifers) and the varying numbers of animals inseminated with semen from each bull (cattle producer's instructions).

10.4.3 Experiment 3

The mean (± SEM) interval from PGF treatment to estrus tended (P = 0.07) to be different among groups (54.9 ± 5.1, 57.0 ± 2.6, and 49.6 ± 1.1 h for the pLH, pLH+P4, and EB+P4 groups, respectively); the variation in this interval was less for the EB+P4 group than for the other two groups (P = 0.002). Estrus rate in the EB+P4 group (15/16, 93.8%) was higher (P < 0.002) than in the pLH Ovsynch group (7/16, 43.8%), while the rate for the pLH Ov-synch+CIDR-B group (12/17, 70.6%) was intermediate and not different from either. The pregnancy rate in the EB+P4 group (75.0%) was also higher (P < 0.04) than in the pLH group (37.5%), while the rate for the pLH+P4 group (64.7%) was intermediate and not different from either (Table 10.1).

10.4.4 Experiment 4

In the Cosynch group, 14 of 17 cows detected in estrus and inseminated between Days 3 and 8 were diagnosed pregnant. The remaining 70 cows were fixed-time inseminated and 39 became pregnant with an overall conception/pregnancy rate to fixed-time AI of 44.8%. In the Cosynch+MGA and EB+MGA groups, pregnancy rates were 47.8% (43/90) and 60.7% (54/89), respectively. The pregnancy rate in the Cosynch and Cosynch+MGA groups tended (P = 0.08) to be lower than in the EB+MGA group (Table 10.1).

Table 10.1 Estrus and pregnancy rates (%) in cattle subjected to different protocols for synchronization of ovulation in fixed-time AI programs.

Experiment	Treatments	n	Animal class	Estrus rate	Pregnancy rate
1	6-day Cosynch	61	Cows	24.6	52.4 (32/61)
	7-day Cosynch	50	Cows	26.0	52.0 (26/50)
2	Cosynch	71	Cows		45.1 (32/71)
	Cosynch+CIDR-B	77	Cows		42.9 (33/77)
	Cosynch	23	Heifers		39.1 (9/23)[a]
	Cosynch+CIDR-B	25	Heifers		68.0 (17/25)[b]
3	pLH Ovsynch	16	Heifers	43.8[c]	37.5 (6/16)[e]
	pLH Ovsynch+CIDR-B	17	Heifers	70.6[cd]	64.7 (11/17)[ef]
	EB/P+CIDR-B	16	Heifers	93.8[d]	75.0 (12/16)[f]
4	Cosynch	87	Cows		44.8 (39/87)[g]
	Cosynch+MGA	90	Cows		47.8 (43/90)[g]
	EB/P+MGA	89	Cows		60.7 (54/89)[h]

Within experiments, values with no common superscripts are different.
[ab] $P < 0.05$, [cd] $P = 0.002$, [ef] $P = 0.04$, [gh] $P = 0.08$

10.5 Discussion

Pregnancy rates in cows synchronized with Cosynch or Ovsynch regimens in Experiments 1, 2 and 4 were similar to those reported previously in lactating beef cows (Geary et al., 2001, Thundathil et al., 1999, Downing et al., 1998, Geary and Whittier, 1997). Furthermore, reducing the interval (from 7 to 6 d) between the first injection of GnRH and PGF treatment did not affect pregnancy rates in the present study. In a previous study (Roy and Twagiramungu, 1999), cows (both lactating and non-lactating) and heifers were given GnRH, followed by PGF 6 or 7 days later, and observed for estrus. It is noteworthy that although a second dose of GnRH was not given and pregnancy rates were not reported, there were no significant differences between the 6- and 7-day regimens for the proportion of cattle in estrus during the interval between GnRH and PGF (8.9 versus 11.8%), or in estrus within 5 days after PGF (53.2 versus 51.3%; Roy and Twagiramungu, 1999). However, the interval from PGF to estrus tended ($P < 0.1$) to be less variable in cattle in the 6-day than in the 7-day regimen (Roy and Twagiramungu, 1999). Although the estrus rate in Experiment 1 was low (approximately 25%), the cows were observed only twice, 48 and 60 h after PGF. Low estrus rates 24 to 72 h after PGF were also reported in nursing cows on 6-day (37.5%) and 7-day (33.8%) GnRH-based protocols (Roy and Twagiramungu, 1999). Data from the present study do not suggest any benefit of one regimen over the other in lactating beef cows.

Combined for Experiments 2 and 3, pregnancy rates in heifers with Cosynch or Ovsynch regimens were 38.5% (n = 39), whereas pregnancy rates were 68.0% (n = 42) for those that also received a CIDR-B device. Low pregnancy rates in heifers have been reported previously in GnRH-based fixed-time AI regimens that did not include a progestin. Pregnancy rates in dairy heifers were 26 to 28% following 7-day Ovsynch protocols (Seguin, 1997, Schmitt et al., 1996a) and 35% following a 6-day regimen in beef cows (Geary and Whittier, 1997). Low pregnancy rates in heifers in Ovsynch and Cosynch regimens may be related to the low incidence of ovulation following the first GnRH treatment and the high percentage of animals that are in estrus before PGF treatment (17.3%; Roy and Twagiramungu, 1999). In this regard, the proportion of heifers ovulating in response to GnRH treatment ranged from 50 to 56% (Pursley et al., 1995, Martínez et al., 2000, Martínez et al., 1999), while the ovulation rate in cows after the first GnRH treatment ranged from 64% (Vasconcelos et al., 1999) to 90% (Pursley et al., 1995).

Synchronous emergence of a new follicular wave (approximately 2 days after GnRH treatment) occurs if the dominant follicle ovulates or if spontaneous wave emergence occurs by chance (Martínez et al., 1999). We hypothesized that the addition of a CIDR-B device to an Ovsynch or Cosynch protocol would improve pregnancy rates to fixed-time insemination by preventing estrus between the first GnRH injection and PGF. In a previous study involving treatment of beef heifers with 100 μg GnRH and insertion of a CIDR-B device at random stages of the estrous cycle (Martínez et al., 2000), GnRH-induced ovulation occurred in only 8 of 16 heifers, but follicular wave

emergence occurred 1.5 ± 0.3 days later (mean ± SEM; range, -1 to 4 days). In that study, the CIDR-B device was removed (and PGF was given) after 6 days but a second dose of GnRH to synchronize the LH surge was not given; ovulation occurred 3.5 ± 0.1 days after CIDR-B removal and conception rate to an insemination 12 h after onset of estrus was 69%. In another study (Section 9.0), heifers treated with a Cosynch+CIDR-B protocol similar to Experiment 2 had pregnancy rates of 65 and 56% in a GnRH- or pLH-based regimen, respectively ($P < 0.6$). Clearly, the inclusion of the CIDR-B device in a GnRH- or pLH-based fixed-time AI program results in synchronous ovulation and acceptable pregnancy rates to fixed-time AI in beef heifers.

The addition of progestins (CIDR-B devices or MGA in Experiments 2 and 4, respectively) to a Cosynch regimen did not significantly improve pregnancy rates in cows. In a previous study (Thompson et al., 1999) in which lactating beef cows were synchronized in an Ovsynch regimen, the pregnancy rate was higher when a norgestomet implant was inserted at GnRH treatment and removed at the time of PGF treatment (10/14, 71.4% versus 4/13, 30.8%, $P < 0.05$). It is noteworthy that cows in that study were only 34.4 days postpartum at the start of the experiment (range, 7 to 44 days) and that the increase in pregnancy rate was attributed to a norgestomet-induced reduction in the incidence of short luteal phases following breeding (Ramírez-Godínez et al., 1981). Stevenson (2000) also reported that pregnancy rates in cows synchronized with an Ovsynch regimen were higher in those receiving a norgestomet implant (51.1%, 47/91 versus 30.7%, 28/91; $P < 0.01$) or a CIDR-B device (66.3%, 63/95 versus 51.1%, 47/92; $P < 0.05$). In these two studies, pregnancy rates in the GnRH-treated controls

were unusually low, but the pregnancy rate in the progestin-treated cows was similar to that in the present study and previous reports (Geary et al., 2001, Thundathil et al., 1999, Downing et al., 1998, Geary and Whitter, 1997). A similar improvement in pregnancy rate was expected in the CIDR-B-treated group in Experiment 2. Although pregnancy rate in the GnRH-treated Control cows was low, the addition of a used CIDR-B device to the protocol did not result in an increase in pregnancy rate. The body condition of the cows at the time of breeding suggested that fertility might be reduced (107/158 had a BCS < 3.0), but there was no significant effect of BCS on pregnancy rate. The semen used was produced commercially and not examined prior to use; semen quality as a limiting factor cannot be ruled out. However, the addition of MGA to a GnRH-based fixed-time AI program in non-lactating beef cows in Experiment 4 also did not result in an improved pregnancy rate. It was concluded that the addition of progestins to a GnRH-based fixed-time AI regimen was beneficial only in heifers.

The incidence of estrus between Days 3 and 8 in the Cosynch group in Experiment 4 (19.5%, 17/87) seemed higher than the incidence of 'early' estrus previously reported for cows (6%; Roy and Twagiramungu, 1996, 1999; 13%, Seguin, 1997). In another report (Downing et al., 1998), cows that were between Days 15 and 17 of their estrous cycle when they were given GnRH came into estrus 11 ± 19 h prior to scheduled PGF treatment (7 days after GnRH). In the present study, the conception rate to estrus detection and AI (82.4%) indicates that the majority of these cows had a true and fertile estrus. If it were assumed that these cows would not be pregnant to the fixed-time AI on Day 9, the pregnancy rate for this treatment group would have been 39/87

(44.8%), which is not significantly different from the pregnancy rate in the Cosynch+MGA group (47.8%). Although cows in the Cosynch+MGA group were not observed for estrus, we speculate that the MGA may have suppressed the expression of estrus in a number of cows that may have subsequently developed persistent follicles (Kojima et al., 1995; Custer et al., 1994) resulting in a reduced pregnancy rate.

In Experiments 3 and 4, pregnancy rates to fixed-time AI were highest for EB-treated cattle. In a previous study utilizing EB in regimens similar to those used in the present experiments, pregnancy rates were 62 and 60% in heifers given a CIDR-B or MGA, respectively (Section 9.0). In another study (Section 7.0), overall pregnancy rate to fixed-time AI was 63% in 84 cows given EB or estradiol-17β (in a 2 x 2 factorial design) in a CIDR-B-based regimen very similar to the one used in Experiment 3. Although the EB+progestin regimens require that the cattle be handled on four occasions (similar to an Ovsynch program but once more than in a Cosynch program), estrogen treatment appeared to induce an estrus more characteristic of a spontaneous estrus (considerable mucus discharge and patent cervical canal) compared to GnRH-treated cattle.

In conclusion, a 6- or 7-day interval from GnRH to PGF in a Cosynch regimen resulted in similar pregnancy rates. The addition of a progestin to a Cosynch or Ovsynch regimen resulted in a significantly improved pregnancy rate in heifers but not in cows. Progestin-based regimens that included EB consistently resulted in high pregnancy rates to fixed-time AI in both heifers and cows.

11.0 THE SYNCHRONIZATION OF FOLLICULAR WAVE EMERGENCE AND OVULATION WITH ESTRADIOL IN A TWO-DOSE PROSTAGLANDIN PROTOCOL FOR FIXED-TIME AI IN BEEF HEIFERS

11.1 Abstract

Prostaglandin has been used in various protocols for estrus synchronization, resulting in conception rates after estrus detection and artificial insemination (AI) equal to or greater than those obtained at spontaneous estrus. However, fixed-time AI after prostaglandin is low. High fertility to fixed-time AI has been achieved in CIDR-B- or MGA-treated heifers given estradiol to synchronize follicle wave emergence and ovulation. The objective of this study was to determine the efficacy of two estradiol formulations for synchronization of follicular wave emergence and ovulation in a two-dose prostaglandin program for fixed-time AI. In Experiment 1, pubertal (confirmed by ultrasound) Angus heifers (n = 561; 13 to 15 mo of age and weighing from 275 to 350 kg) received 500 μg im of cloprostenol (PGF) twice (Days 0 and 14) and were assigned to 4 groups in a 2 x 2 factorial design. On Day 7, half were given 2 mg im estradiol benzoate (EB) and 50 mg progesterone (P) in canola oil (EBP). The other half received no treatment. Twenty-four hours after the second PGF injection (Day 15), the two groups were subdivided to receive 1 mg im EB in canola oil or no treatment. All heifers were observed for estrus twice daily from Days 13 to 17. Heifers treated with EB on

Day 15 were fixed-time inseminated 52 h after PGF (28 h after EB). Heifers detected in estrus more than 12 h before the appointed breeding time and those not given EB on Day 15, were inseminated 4 to 8 h after estrus signs were first detected. All heifers not inseminated by 72 h after PGF were given 100 μg im GnRH and were concurrently inseminated. Ultrasonographic pregnancy diagnosis was done 30 to 35 d after AI. The overall pregnancy rate was higher in heifers that received EBP than in those that did not (61.6 vs 48.2%, respectively). Pregnancy rate was lower in heifers that received EB after PGF than those that did not, (50.0 vs 59.8%; P = 0.02). However, 52 of 279 (19%) heifers treated with EBP were detected in estrus early and presumably would not have conceived to the fixed-time insemination. Although treatment with EBP on Day 7 apparently induced premature luteolysis in these heifers, they were inseminated shortly after they were detected in estrus (and the conception rate was 44.2%). Notwithstanding, treatment with EBP on Day 7 resulted in a conception rate of 58.1% after GnRH given 72 h after the second PGF treatment with fixed-time AI concurrently. In Experiment 2, pubertal Angus heifers (n = 401) with a CL (confirmed by ultrasonography) received 500 μg im PGF on Day 0 (beginning of the experiment) and Day 14. Heifers were assigned to 4 groups in a 2 x 2 factorial design. On Day 7, heifers received either no further treatment (Control) or 1.5 mg estradiol-17β (E-17β) and 50 mg P in canola oil im. On Day 15 (24 h after the second PGF), heifers received either no further treatment or 1 mg E-17β in canola oil im, with fixed-time AI 28 h later (52 h after PGF). Heifers that received no treatment after PGF were observed for estrus from Days 14 to 17 and inseminated accordingly (4 to 8 h later). Heifers, not in estrus by 72 h, were given 100 μg GnRH im and concurrently inseminated. Pregnancy was diagnosed by

ultrasonography 30 d after AI. Estrus rate during the first 72 h after the second PGF treatment was higher ($P < 0.05$) in the E-17βP group than in the Control group. Pregnancy rates to detected estrus were not different ($P = 0.5$) between the two groups not receiving E-17β after the second PGF (51 vs 49% for NT/NT and E17β/NT, respectively). The group treated only with E-17β on Day 15 tended to have the lowest ($P < 0.06$) proportion of heifers pregnant to fixed-time AI (39.0%), while the E-17βP group treated with E-17β on Day 15 had a pregnancy rate of 48.5% to fixed-time AI. There was no significant difference among the other treatment groups. In summary, synchronization of follicular wave emergence and ovulation in a two-dose PGF-based protocol resulted in acceptable fertility to fixed-time AI. Although EB and E-17β were not directly compared, both resulted in acceptable pregnancy rates to fixed-time AI after synchronization of follicular wave emergence and ovulation. In particular, when E-17βP was used to synchronize follicular wave emergence, premature estrus was not induced. Further research is necessary to assess time of ovulation after manipulation of follicular growth with E-17β and progesterone in PGF-based synchronization programs for fixed-time AI.

11.2 Introduction

Prostaglandin F2α (PGF) has been widely used for estrus synchronization (Odde, 1990; Larson and Ball, 1992). Originally, reports of a single dose, given as an intrauterine infusion (Rowson et al., 1972), intravenous, subcutaneous (Lauderdale, 1972) or intramuscular injection (Roche, 1974) effectively induced luteolysis, followed

by behavioural estrus. When PGF treatment was given between Days 1 and 4 of the estrous cycle, it did not affect corpus luteum (CL) lifespan (Lauderdale, 1972; Rowson et al., 1972; Momont and Seguin, 1984). Although PGF is very effective in inducing luteolysis from Days 6 to 16 of the estrous cycle, there is considerable variability in the interval from treatment to estrus and ovulation (Momont and Seguin, 1984). Early studies were designed to ensure the presence of a susceptible CL, either by administering a single injection PGF after detecting a CL by rectal palpation or giving two injections of PGF, 11 or 14 d apart (Larson and Ball, 1992). Although fertility has been reported to be high, PGF-based protocols did not provide sufficient synchrony for fixed-time AI (Peters, 1986).

Preliminary studies were designed to investigate follicular wave patterns and the variation in the interval from PGF administration to estrus (Macmillan and Henderson, 1984). The effect of day of PGF treatment on the selection and development of the ovulatory follicle was investigated by Kastelic et al. (1990a). When PGF was given on Days 5 or 8 after ovulation, the dominant follicle of the first follicular wave ovulated in 2 or 3 days, whereas PGF administration on Day 12 after ovulation resulted in ovulation of the dominant follicle of the second follicular wave 5 days later. Therefore, the length of the interval from PGF treatment to estrus and ovulation depends on the stage of development of the dominant follicle at the time of treatment; this is the basis of the variability in the interval from treatment to estrus and ovulation in cattle treated at random days of the estrous cycle.

Estrogens have been used to increase the synchrony of estrus in PGF-based estrus synchronization programs. Treatment with 0.4 mg estradiol benzoate (EB) 40 or

48 h after PGF administration to cyclic beef cows reduced the variation in the interval from PGF to LH release and onset of estrus (Welch et al., 1975). In another study, this protocol tended to increase the proportion of lactating dairy cows in estrus within 5 d after PGF treatment (Dailey et al., 1986). However, it did not affect estrus rate in dairy heifers (Dailey et al., 1986) or beef cows and heifers (Peters et al., 1977). In these studies, synchronization of estrus and ovulation was attempted without synchronization of follicular development; and thus, there were no effects on pregnancy rates; fixed-time AI was not investigated.

More recently, treatments to Control the timing of follicular wave emergence, such as estradiol or estradiol plus progesterone combinations (Bó et al., 1995a), GnRH (Twagiramungu et al., 1995; Pursley et al., 1995; Thatcher et al., 1993; Kastelic and Mapletoft, 1998), pLH (Section 10.0), or hCG (Schmitt et al., 1996a), have been incorporated into estrus synchronization programs with the objective of synchronizing the recruitment of a growing dominant follicle. Approximately 4 to 5 d after emergence of a new follicular wave, PGF treatment was administered and progesterone was removed, and the dominant follicle ovulated approximately 3 to 4 d later (Martínez et al., 2000). In these protocols, ovulation of the dominant follicle is often synchronized by a second treatment with estradiol (sections 6.0, 9.0), GnRH (sections 9.0, 10.0), pLH (sections 9.0, 10.0) or hCG (Schmitt et al., 1996a), followed by a single, fixed-time insemination.

High fertility to fixed-time AI has been achieved in CIDR-B- or MGA-treated heifers given EB (EB) to synchronize follicle wave emergence and ovulation (sections 5.3, 6.0, 7.0). The manipulation of follicular growth and ovulation with estradiol in a

traditional two-dose PGF protocol has not yet been studied. The objective of this study was to determine the efficacy of estradiol treatments administered to synchronize follicular wave emergence and ovulation for fixed-time AI in a two-dose PGF protocol.

11.3 Materials and methods

11.3.1 Experiment 1

Pubertal Angus heifers (n = 561; 13 to 15 mo of age and 275 to 350 kg) with a CL (confirmed by ultrasonography) that had been in a feedlot for approximately 7 mo and were fed approximately 5 kg barley silage and 1 kg of rolled barley/animal/d were used. Heifers received 500 μg im of cloprostenol (PGF, Estrumate; Schering-Plough, Pointe Claire, PQ, Canada) on Day 0 (beginning of the experiment) and Day 14, and were assigned to 4 groups in a 2 x 2 factorial design. On Day 7, half of the heifers were assigned to receive no treatment (Control group) and half received 2 mg estradiol benzoate (EB) and 50 mg progesterone (P; both from Sigma-Aldrich Canada Ltd, ON, Canada) in canola oil im (EBP group). Half of the heifers in each group received 1 mg im EB in canola oil 24 h after the second PGF (Day 15). All heifers were observed for estrus twice daily from Days 13 to 17. Heifers treated with EB on Day 15 were fixed-time inseminated 28 h later (52 h after PGF). Heifers detected in estrus more than 12 h before the appointed breeding time and those not given EB on Day 15 were inseminated 4 to 8 h after onset of estrus. All heifers not inseminated by 72 h were given 100 μg im GnRH (Cystorelin; Merial Can. Inc, Victoriaville, PQ, Canada) and concurrently inseminated. Pregnancy diagnosis was performed by ultrasonography 30 to 35 d after

AI. Conception rate was calculated as the number of heifers that became pregnant from the total of heifers that were inseminated. Pregnancy rate was calculated as the number of the heifers that became pregnant As the data were considered to be coming from four different treatment protocols with two of them including GnRH, which modified the original 2x2 factorial design, a Chi-square test was used to compare proportion of pregnant animals. Proportional data were compared among groups by Mantel-Haenszel Chi-square test and the interval from the second PGF treatment to estrus was analyzed by two-way analysis of variance. Bartlett's test was used to test homogeneity of variances (Norman and Streiner, 2000). All statistical analyses, except Mantel-Haenszel test, were conducted with a current statistical program (Statistix Student Version, version 2.0, Analytical Software, Tallahassee, Florida, USA).

11.3.2 Experiment 2

Pubertal Angus heifers (n = 401; similar age and weight and in the same housing conditions as in Experiment 1) with a CL (confirmed by ultrasonography) received 500 μg im PGF on Day 0 (beginning of the experiment) and Day 14, and were assigned to 4 groups in a 2 x 2 factorial design. On Day 7, half of the heifers were assigned to receive no treatment (Control group) and the other half received 1.5 mg estradiol-17β (E-17β; Sigma-Aldrich Canada Ltd) and 50 mg P in canola oil im. Half of the heifers in each group received 1 mg im E-17β in canola oil 24 h after the second PGF (Day 15). All heifers were observed for estrus twice daily from Days 14 to 17. Heifers treated with E-17β on Day 15 were fixed-time inseminated 28 h later (52 h after PGF). Heifers detected

in estrus more than 12 h before the appointed breeding time were inseminated 4 to 8 h after first estrus detection. All heifers not inseminated by 72 h received 100 μg im GnRH and were concurrently inseminated. Pregnancy was diagnosed by ultrasonography 30 to 35 d after AI. Statistical analyses were performed the same as in Experiment 1.

The protocols for these experiments were approved by University of Saskatchewan Animal Care Committee.

11.4 Results

11.4.1 Experiment 1

Results are shown in Tables 11.1 and 11.2. There were 52 of 279 heifers treated with EBP (19%) that were detected in estrus more than 12 h before the appointed AI time (Table 11.1) and they were considered as non-pregnant to fixed-time AI. Estrus rate differed among groups ($P = 0.01$); in the heifers treated with EBP, those also treated with EB were 1.65 times more likely to be in estrus than those that were not. There was no effect of treatment on the proportion of heifers detected in estrus that became pregnant ($P = 0.16$). However, when groups were pooled by treatment, pregnancy rate was higher in heifers that received EBP, whether or not they received EB after PGF (61.6 vs 48.2%, $P < 0.001$). Pregnancy rate was lower in heifers that received EB after PGF, regardless of whether they received EBP (50.0 vs 59.8%, $P = 0.02$).

Table 11.1 Estrus and conception rates in Control (NT/NT) heifers or those treated with estradiol benzoate (EB) and progesterone (P) to synchronize follicular wave emergence and/or EB to synchronize of ovulation in a two-injection, PGF-based program for fixed-time AI.

Treatment	NT/NT	EBP/NT	NT/EB	EBP/EB
No. of heifers	142	139	140	140
No. of heifers in estrus (Conception rate (%)				
Pre-second PGF (Day 14)	0 -	11 (27.3)	0 -	18 (22.2)
Post-second PGF (Day 14)				
12-24 h	6 (0.0)[a]	15 (73.3)[b]	0 -	8 (62.5)[b]
36-48 h	39 (64.1)[a]	25 (72.0)[a]	89 (47.2)[b]	73 (58.9)[ab]
60-72 h	33* (72.7)	45* (84.4)	0 -	0 -

[ab] Within a row, values with different superscripts are different ($P < 0.05$).
* These heifers were treated with 100 μg GnRH 72 h after the second injection of PGF and concurrently fixed-time inseminated.

Table 11.2 Estrus, conception, and conception rates (%), and mean (± SEM) interval from the second PGF treatment to estrus in Control (NT/NT) heifers or those treated with estradiol benzoate (EB) and progesterone (P) to synchronize follicular wave emergence and/or EB to synchronize of ovulation in a two-injection, PGF-based program for fixed-time AI.

	NT/NT	EBP/NT	NT/EB	EBP/EB
No. of heifers	142	139	140	140
Interval PGF to estrus (h)	$50 \pm 1.2^{a\ x}$	$48 \pm 1.4^{ab\ x}$	$45 \pm 0.4^{bc\ y}$	$42 \pm 0.8^{c\ z}$
Estrus rate*	54.9	69.1	63.6	70.7
Conception rate in estrous heifers	62.8	72.9	47.2	52.5
No. of heifers not in estrus	64	43	51	41
Conception rate in non-estrous heifers	37.5	58.1	41.2	61.0
Conception rate to fixed-time AI**	37.5^{a}	58.1^{b}	45.0^{ab}	48.6^{ab}
Overall pregnancy rate	51.4^{a}	68.3^{b}	45.0^{a}	55.0^{a}

^{ab} Means and ^{xyz} variances with different superscripts are different ($P < 0.05$).
* Detection of estrus until 72 h after the second dose of PGF.
** Pregnant to the fixed-time AI done at the same time as treatment with GnRH at 72 h after the second PGF in the heifers that received no treatment following the second PGF injection or to the fixed-time AI at 28 h after 1 mg EB (52 h after the second PGF treatment) without regard to estrus. Heifers that showed estrus more than 12 h before the fixed-time for AI in the EBP/EB group were considered as non-pregnant to fixed-time AI.
NT = no treatment.

11.4.2 Experiment 2

Results are shown in Table 11.3. Estrus rate during the first 72 h after the second PGF treatment was higher ($P < 0.05$) in the E-17βP/NT group than in the Control group; the other two groups were not observed for signs of estrous behaviour. Pregnancy rates in the two groups in which estrus detection was done were not different ($P = 0.5$). Although pregnancy rates to fixed-time AI did not differ ($P = 0.28$) among groups, the NT/E-17β group had the least number of heifers pregnant to fixed-time AI.

Table 11.3 Estrus, conception and conception rates (%) in Control (NT/NT) heifers or those treated with estradiol-17β (E-17β) and progesterone (P) to synchronize follicular wave emergence and/or E-17β to synchronize ovulation in a two-injection, PGF-based program for fixed-time AI.

Treatment	NT/NT	E-17βP/NT	NT/E-17β	E-17βP/E-7β
No. of heifers	100	100	100	101
Estrus rate	16[a]	45[b]	ND	ND
Conception rate in estrous heifers	50.0	60.0	--	--
Conception rate to fixed-time AI*	51.2	40.0	39.0	48.5
Overall pregnancy rate	51.0[c]	49.0[cd]	39.0[d]	48.5[cd]

Percentages with different superscripts differed [ab] ($P < 0.05$); [cd] ($P = 0.065$).
NT = no treatment; ND = not determined.
* Heifers of Control and E-17βP/NT groups that were not detected in estrus were fixed-time inseminated and concurrently given 100 μg GnRH 72 h after the second PGF treatment, whereas heifers treated with E-17β 24 h after the second PGF treatment were inseminated 28 h later (52 h after PGF).

11.5 Discussion

To our knowledge, the use of the combination of estradiol and progesterone to synchronize follicular wave emergence, and estradiol to induce ovulation in a 2-injection PGF-based synchronization program for fixed-time AI, has not previously been reported. Pregnancy rates to detected estrus or fixed-time AI with both estradiol formulations in the two experiments were acceptable, especially when follicular wave emergence was synchronized.

Estradiol-17β has been used to synchronize follicular wave emergence in progestin-treated beef heifers (Bó et al., 1994, 1995a,b). However, in the absence of endogenous and exogenous progestins, E-17β induced an LH surge that was associated with rescue of the dominant follicle present at the time of treatment (Bó et al., 1994). Based on previous studies, the interval from the first PGF treatment at random stages of the estrous cycle to ovulation varied by at least 3 d (Kastelic et al., 1990a). Therefore, at the time of estradiol plus progesterone treatment on Day 7, these heifers would have been at varying stages of metestrus or early diestrus. As circulating progesterone concentrations may have not been sufficiently high to prevent an estradiol-induced LH surge, progesterone was injected along with estradiol. In other studies in our laboratory, we have successfully used the combination of 100 mg progesterone with 5 mg E-17β or 1 mg EB to synchronize follicular wave emergence at random stages of the estrous cycle (Section 6.0).

When 2 mg EB was administered on Day 7 in Experiment 1, the expression of estrus in heifers prior to the second PGF and the increased variability of the interval from the second PGF to estrus were attributed to hastening uterine-induced luteolysis. These heifers were inseminated based on behavioural estrus and were considered not pregnant to fixed-time AI. The dose of EB used had been derived from reports that suggested that an intermediate dose of 2.5 mg of EB induced a more synchronous emergence of the next follicular wave (in CIDR-B-treated cattle) than either 1 mg or 5 mg EB (Caccia and Bó, 1998). However, in this study the CIDR-B devices would have prevented animals from expressing estrus and ovulating, even though the EB may have induced luteolysis. Cows used had greater body mass and hormone-metabolizing capacity that may have resulted in EB not inducing luteolysis.

Estrogens have been shown to have luteolytic properties when given alone or in association with progestins (Wiltbank et al, 1961; Wiltbank and Kasson, 1968; Munro and Moore, 1985). Treatment of beef heifers on Days 3, 10 or 17 after estrus with 5 mg EB decreased blood progesterone concentrations from 2 to 5 d after treatment (Munro and Moore, 1985). The intravaginal use of the crystalline form of EB (10 mg; slow release) has also been reported to induce luteal regression in progestin-based estrus synchronization programs (Macmillan et al., 1991; Burke et al., 1999). However, Burke et al. (2000) have also shown that a dose of 1 mg EB given im on Day 13 of the estrous cycle in Holstein cows caused a decline in circulating progesterone concentrations between 24 and 48 h after treatment and hastened regression of the CL. Therefore, even a small dose of EB may affect progesterone profiles even though luteolytic effects increase when the dose of EB is increased (as in our experiment).

Based on the assumption that EB treatment would induce premature luteolysis in some heifers, 1.5 mg E-17β was administered (along with progesterone) on Day 7 to synchronize follicular wave emergence in Experiment 2. Characteristics of plasma estradiol concentrations after E-17β treatment are different from those after EB treatment, as confirmed by studies in both ovariectomized (section 5.0) and intact (Bó et al., 2000a) cattle. Plasma estradiol concentrations reached a higher peak and declined more rapidly after an injection of E-17β as compared to the same dose of EB (section 5.0). When 1 mg E-17β was given to norgestomet-treated heifers, emergence of the next follicular wave occurred earlier than in Control heifers (Bó et al., 2000a). Doses less than 1 mg E-17β were considered to have an inconsistent effect in dominant follicle suppression (Bó et al., 2000a). In Experiment 2, luteolysis apparently did not occur after the administration of E-17β, as there were no heifers detected in behavioural estrus before the second treatment with PGF on Day 14 (or even before the second E-17β treatment on Day 15).

The use of estradiol on Day 15 not only resulted in an increased proportion of cattle expressing estrus, but also reduced the variability in the interval to estrus in both experiments. It has been reported that EB treatment reduced the variation in the time of LH release and the onset of estrus, and increased the number of cattle detected in estrus within a target period of 56 to 86 h after PGF injection (Welch et al., 1975). However, conception rates remained similar to those in the animals not receiving EB (Peters et al., 1977; Welch et al., 1975). The administration of estradiol following a two-dose prostaglandin protocol would appear to only synchronize ovulation when follicular wave emergence had also been synchronized i.e., a mature dominant follicle was present at the

time of treatment. If the emergence of follicular waves is not synchronized, the probability of synchronous ovulation as a result of a large dominant follicle responding to the estradiol-induced LH surge will be very much reduced resulting in poor pregnancy rates to fixed-time AI. When follicular waves and ovulation were synchronized, pregnancy rates to fixed-time AI were comparable to Control groups with AI after estrus detection.

Previous studies have shown that fixed-time AI 72 and 96 h after the second PGF injection in a two-dose treatment protocol resulted in calving rates (43%) that were lower than in untreated Controls inseminated at detected estrus (49.1%; Roche and Prendiville, 1979). In another trial, pregnancy rate in cows following a two-injection PGF treatment protocol with fixed-time AI at 72 and 96 h (31.6%) was poor compared to that in cows treated with a single dose of PGF and inseminated at estrus (47.8%; Roche and Prendiville, 1979). More recently, Slenning (1992) reported a low (21%) pregnancy rate following a single insemination by appointment after PGF treatment and when GnRH treatment, was administered at 48 h with insemination 16 to 20 h later in a two-dose PGF regime, pregnancy rate was also low (27%). In the present study, two doses of PGF 14 d apart, followed by GnRH and AI at 72 h resulted in acceptable pregnancy rates (37.5 and 51.2%; Experiments 1 and 2, respectively). The interval of 72 h after the second PGF provided sufficient time for further growth of dominant follicles emerging spontaneously in cattle in which follicular wave emergence was not synchronized. The combination of GnRH and fixed-time AI 72 h after the second PGF could be an interesting alternative to maintain acceptable fertility in a two-PGF

synchronization protocol, avoiding additional estrus detection and AI for longer periods of time.

In summary, fixed-time AI was successful in a PGF-based synchronization program in which follicular wave emergence and ovulation were synchronized. It is noteworthy that conception rates to fixed-time AI were consistently high when E-17β was used, whereas EB apparently induced luteolysis in some animals and effective synchronization of estrus and ovulation was not achieved. Although EB and E-17β were not directly compared, both resulted in acceptable pregnancy rates to fixed-time AI after synchronization of follicular wave emergence and ovulation. Further studies are needed to determine the most appropriate dose of EB, which will effectively synchronize follicular wave emergence in all cattle without causing premature luteolysis. Results clearly showed that synchronization of follicular wave emergence was necessary if the objective was the use of an ovulation synchronization program for fixed-time AI in PGF-treated cattle.

12.0 GENERAL DISCUSSION

In the last 60 years, AI has relied on visual estrus detection. Using this method, cattle are observed at different times during the day for signs of estrus and inseminated within 12 h of standing estrus. The most common schedule of AI in a herd has been the AM-PM rule, in which cattle detected in estrus in the morning are inseminated the afternoon of the same day and cattle detected in the afternoon are inseminated the next morning. The laborious and tedious task of estrus detection has an efficiency, which has been reported at 50% or less (Stevenson and Britt, 1977; Nebel, 2000). Poor estrus detection efficiency also results in the insemination of cattle with high circulating progesterone concentrations (i.e., 19%; Sturman et al., 2000). In an extensively managed beef cattle operation, it is necessary to bring the cattle from the pasture, sort cows from calves, and sort those detected in estrus to be inseminated. Estrus detection and sorting are performed repeatedly in beef cattle herds throughout the breeding season.

Traditional methods of estrus synchronization (such as two-dose of PGF 11 or 14 days apart) can reduce the number of days that estrus detection must be performed. However, variation of the interval from the last PGF treatment to estrus remains substantial (approximately 2 to 7 days). Both the required management and the inaccuracy of visual estrus detection contribute to the lack of interest in the use of AI by beef cattlemen. The use of AI is very low in beef cattle (5% of Canadian beef herds), especially when compared to the dairy industry (70% of Canadian dairy herds). The use of AI will be become increasingly attractive to beef producers with the availability of

new protocols that involve fewer treatments, less handling and sorting, avoid estrus detection, facilitate fixed-time AI, and result in high pregnancy rate.

Fixed-time AI has been used since the 1970's. Using a two-dose PGF-based protocol with double inseminations at 72 and 96 h after the second PGF treatment, conception rates were similar to those in Control cattle with AI after estrus detection (Lauderdale, 1975). However, later reports suggested that this protocol did not result in acceptable pregnancy rates (reviewed by Peters, 1986; Larson and Ball, 1992). Therefore, the protocol of two PGF injections 11 to 14 days apart followed by two inseminations was not adopted as a regular synchronization program (Peters, 1986). In addition, two inseminations considerably increased the cost of the AI program. The combinations of progestins with PGF or progestins with estradiol as a luteolytic agent for timed AI have also been used, but again pregnancy rates have been less than satisfactory (Larson and Ball, 1992).

More recently, ultrasonographic imaging of the ovaries has increased knowledge of follicular and luteal dynamics in cattle (Pierson and Ginther, 1984). Ultrasound monitoring of ovaries has enabled the identification of different stages of follicular development as the cause of variability in estrus and ovulation after PGF treatment (Kastelic et al., 1990a). In addition, it was determined that the low fertility reported after progestin-based synchronization protocols (Odde, 1990) was due to the development of persistent follicles that ovulated aged oocytes (Smith and Stevenson, 1995; Cooperative Regional Research Project, 1996). Effects of hormonal (estradiol, progesterone, GnRH, LH) or mechanical (follicular ablation) treatments on the follicular population have been also investigated (Macmillan and Thatcher, 1991; Bó et al., 1995a; Bergfelt et al. 1994;

Twagiramungu et al., 1995; Martínez et al., 1999). Treatments that resulted in atresia (estradiol and progesterone) or ovulation (GnRH or LH) of the dominant follicle (including persistent follicles) induced the emergence of a new follicular wave. These treatments also reduced, in part, the variation in the interval from PGF treatment to estrus and to ovulation (reviewed in Section 2.0). Based on these results, a new generation of protocols has been developed with acceptable fertility to AI at detected estrus (Twagiramungu et al., 1992; Bó et al., 1995a; Martínez et al., 2000). Although protocols have been designed to synchronize estrus and ovulation (reviewed in Section 2.0), a treatment for the synchronization of both follicular wave emergence and ovulation was not included in the same protocol.

The recently developed GnRH-based Ovsynch (ovulation synchronization) program involves two GnRH treatments; the first treatment (given 7 days before PGF injection) is to synchronize follicular emergence, while the second treatment (2 days after PGF injection) is to induce ovulation of the newly recruited dominant follicle. Therefore, this protocol allows for the use of fixed-time AI. Results obtained after the use of this protocol in dairy (Pursley et al., 1995) and beef (Geary et al., 1998) cattle were promising (the need for estrus detection was eliminated and fertility to fixed-time AI acceptable was acceptable). However, Ovsynch protocols have not been effective in heifers, which has been attributed to the failure of the first GnRH treatment to induce ovulation and/or synchronization of follicular wave emergence. The Ovsynch protocol has resulted in pregnancy rates to fixed-time AI that have varied from 32 to 55% in lactating dairy cattle (Pursley et al., 1995, 1997; Jemmeson, 1998) and from 40 to 53% in lactating beef cattle (Geary et al., 2001; Small et al., 2001), whereas pregnancy rates

to the same program in heifers varied from 25% (Schmitt et al., 1996b) to 35% (Pursley et al., 1997). Based on these results, there was still a need for the development of new protocols that not only included fixed-time AI, but also resulted in pregnancy rates comparable to AI after estrus detection in cattle in all physiological states. The objective of an ovulation synchronization program for fixed-time AI is to ensure the presence of a healthy dominant follicle at the time of treatment for ovulation, which may then be induced to ovulate within a short interval. Upon ovulation, a healthy oocyte will be ready for fertilization by a single insemination at a predetermined time. Therefore, a protocol for ovulation synchronization requires control of both the luteal phase and follicular growth (synchronization of follicular wave emergence) as well as the induction of ovulation after progesterone removal.

The overall objective of the work reported in this thesis was the development of new methods of synchronization of ovulation to facilitate fixed-time AI in cattle. Since control of follicular growth plays an important role in these methods, different approaches to the synchronization of follicular wave emergence were investigated. A detailed description of the effect of the most successfully applied techniques of follicular wave synchronization have been presented, as well as the effect of estradiol, GnRH, and LH treatments on fertility after fixed-time AI. In addition, synchronization programs based on progestins and PGF were examined.

12.1 Effects of estradiol and progesterone on follicular development for synchronization protocols.

Ovarian steroid hormones (estradiol and progesterone) influence the synthesis and secretion of pituitary gonadotrophins (LH and FSH). During the bovine estrous cycle, gonadal steroid production is characterized by high progesterone and low estradiol concentration in the circulation, while at the end of the estrous cycle, the CL regresses and the dominant follicle of the last follicular wave becomes preovulatory, secreting increasing amounts of estradiol. Therefore, the proestrous period is characterized by declining concentrations of progesterone and increasing concentrations of estradiol. As a result, LH pulse frequency increases, concluding in an LH surge concurrently with the onset of estrus. Estrogens (from follicles) and progesterone (from the CL) influence gonadotrophin secretory patterns during the estrous cycle and also affect follicle growth and development of the CL.

The use of ovarian steroids to manipulate follicular development has been reviewed (Section 2.0) and reported in this thesis. Previous studies showed that the combination of 5 mg E-17β and progestin implants synchronized the emergence of a new follicular wave (Bó et al., 1995a). We have also observed that the effect of estradiol on gonadotrophins and follicular wave emergence depended on the dose and form of estradiol used. Treatment with 1 mg E-17β resulted in the emergence of a new follicular wave with more variability (3 to 5 days after treatment) than 5 mg E-17β (3 to 4 days after treatment) in CIDR-B treated cows (Section 6.0). In contrast, treatment with 1 mg EB resulted in the emergence of a new follicular wave with less variability than 5 mg EB (Section 6.0). In another experiment (Section 6.0), treatment with 5 mg E-17β or 1

mg EB given along with 100 mg progesterone at CIDR-B insertion in beef heifers resulted in a new follicular wave 3 to 4 and 3 to 5 days later, respectively. Burke et al. (2001) observed that 1 mg EB 500 Kg^{-1} body weight (BW) resulted in a mean interval of 3.1 ± 0.1 days from treatment to the emergence of a new follicular wave. These studies showed that the dose and form of estradiol were important aspects to consider in the synchronization of follicular wave emergence in CIDR-B treated cattle.

It was hypothesized that the effect of estradiol and progestins on the follicle development consisted of two principal mechanisms: 1) suppression of follicles, and 2) induction of emergence of a new follicular wave. The first mechanism would depend on estradiol suppressing plasma FSH concentrations causing regression of follicles prior to the time of selection of the dominant follicle, whereas the suppression of plasma concentrations and pulsatility of LH by progesterone would be associated with the regression of the selected dominant follicle which has been reported to occur (3 days after wave emergence, Ginther et al., 1996b). The second mechanism (induction of follicular wave emergence) would depend only on the decline of plasma estradiol concentrations, which would result in resurgence of plasma FSH concentrations in a surge-like manner and emergence of a new follicular wave.

One of the proposed suppressive mechanisms of estradiol treatment was the inhibition of follicular growth by suppression of FSH, before selection of the dominant follicle. Treatment with 5 mg E-17β in ovariectomized beef cows resulted in suppression of plasma FSH concentrations within 6 h, with resurgence after 24 h, reaching concentrations similar to pre-treatment levels at 48 h after the estradiol injection (Section 5.0). A similar profile in plasma FSH concentrations was achieved

after an injection of 5 mg E-17β in progestin-treated intact heifers (Bó et al., 1994) or after the injection of 1 mg of EB in ovariectomized cattle (Section 5.0, O'Rourke et al., 2000). Since 5 mg E-17β and 1 mg EB resulted in similar FSH profiles, these treatments may result in similar synchrony of follicular wave emergence, similar to that obtained in intact heifers (Section 6.0). It has also been documented that estradiol treatment in ovariectomized heifers resulted in decreased plasma FSH concentrations (Price and Webb, 1988). In intact animals, after treatment with estradiol, follicles do not reach a diameter greater than 8 mm (size at follicular growth divergence) and go to atresia, as demonstrated by the reduction in diameter during the subsequent days (Bó et al., 1994). There is clear evidence that this suppression is exerted via reduction of plasma FSH concentrations, which affect FSH-dependent follicles.

The mechanism of progesterone-induced suppression of follicular growth is apparently through inhibition of pituitary secretion of LH that resulted in atresia of a selected dominant follicle. In intact animals, plasma LH concentrations are influenced by progesterone secretion by the CL. High concentrations of progesterone during mid-luteal phase were shown to inhibit LH release; however, plasma progestin concentrations after treatments at recommended doses for estrus synchronization did not appear to cause the same suppression on LH release (Kojima et al., 1995). It has also been shown that treatment with progesterone-impregnated devices reduced LH pulse frequency and mean plasma LH concentrations in ovariectomized heifers (Price and Webb, 1988).

In our studies in ovariectomized cows, progesterone from the CIDR-B devices suppressed plasma LH concentrations from 36 to 72 h after insertion, when the stimulatory effect of estradiol (given at the beginning or one day after the CIDR-B insertion) on LH disappeared (Section 5.0). Other studies have shown that daily injections of progesterone in intact heifers caused atresia of the dominant follicle in a dose-dependent manner (Adams et al., 1992b). An injection of 100 mg progesterone in oil or in saline, or a CIDR-B device for 24 h in norgestomet-implanted Zebu heifers also resulted in suppression of the dominant follicle present at treatment and reduced variability in the interval to the emergence of the ovulatory wave compared to the Control group receiving no treatment (Cavalieri et al., 1998). It is noteworthy that follicular waves emerged earlier in heifers treated with CIDR-B devices than in those treated with an injection of progesterone (Cavalieri et al., 1998). The effect of CIDR-B on follicular dynamics was probably due to a longer duration of elevated plasma progesterone concentrations, whereas a progesterone injection would result in a peak followed by a continuous decline. In addition, 200 mg progesterone resulted in increased plasma progesterone concentrations more than 1 ng/mL in heifers during the follicular phase of the estrous cycle and regression of persistent follicles in MGA-treated cattle (Anderson and Day, 1994). In our studies, a single injection of 100 mg progesterone in ovariectomized cows increased plasma progesterone concentrations by 2 ng/mL over 24 h, but caused no decrease in plasma LH concentrations (Section 5.0), probably due to a strong effect of estradiol injected concurrently. Insertion of a CIDR-B device appeared to have an extended effect on plasma LH concentrations (~72 h after insertion).

It has been shown that LH receptor mRNA is expressed in granulosa cells of dominant follicles only after Day 3 following ovulation (Xu et al., 1995), around the time selection of a dominant follicle occurs (Ginther et al., 2000). Therefore, the dominant follicle becomes LH-dependent and plasma LH concentrations may be critical to maintain dominance. As an example of dominant follicle regression, the insertion of a CIDR-B device in beef heifers resulted in the emergence of a new follicular wave 2 to 5 days later in 72% of the heifers (Martínez et al., 2000). If there were heifers with an LH-dependent dominant follicle, the steady increase and maintenance of plasma progesterone concentrations by CIDR-B devices may have affected circulating LH concentrations at least for the first 3 days after insertion as occurred in ovariectomized cows, (Section 5.0). This suppression of plasma LH concentrations caused atresia of the dominant follicle, resulting in an FSH surge and emergence of a new follicular wave.

Our results in Section 5.0 and previous results reported by Macmillan et al. (1991) showed that progesterone release from a CIDR-B device over the first 3 days after insertion was equivalent to that reported between days 11 and 15 of the estrous cycle (Kesner et al., 1982). Injection of 100 mg progesterone resulted in a significant increase in plasma progesterone concentrations in CIDR-B-treated cows or those that did not receive a CIDR-B device (Section 5.0). However, the increase in plasma progesterone in both groups of cows was not sufficient to prevent the LH surge induced by the treatment with 5 mg E-17β. It has been also reported that doses ranging from 0.25 to 10 mg E-17β induced LH release in ovariectomized cows that received exogenous progesterone given in the form of implants 2 to 9 days before the estradiol treatment (Short et al., 1973). In the same study, it was shown that the estradiol-induced LH surge

was prevented in progesterone-treated intact cattle with >1 ng/mL plasma progesterone. These results in intact cattle were confirmed by a study in which an injection of 5 mg E-17β did not result in an LH surge in heifers that received a norgestomet implant one day prior to treatment (Bó et al., 1994). The differences in the responses obtained in these experiments may be explained by the status of cattle, i.e., ovariectomized (Section 5.0) vs intact (Bó et al., 1994).

The second mechanism that contributes to emergence of the next follicular wave after treatment with estradiol and progesterone was attributed to the FSH surge, which depended on the duration of plasma estradiol concentrations. After treatment with 5 mg E-17β in CIDR-B treated cattle, at random stages of the estrous cycle, a new follicular wave emerged 3 to 4 days later (Section 6.0). When 5 mg E-17β was administered in CIDR-B-treated ovariectomized cows, plasma estradiol concentrations decreased to baseline 36 h after treatment; this resulted in resurgence of plasma FSH concentrations to pretreatment concentrations by 42 to 48 h after treatment (Section 5.0). Similar profiles of plasma FSH concentrations were also observed after injection of 5 mg E-17β in intact heifers (Bó et al., 1994). It has been reported that FSH surges occur one day before the emergence of a follicular wave in estradiol-treated females (Bó et al., 1994), as has been observed in spontaneous follicular waves during the estrous cycle (Adams et al., 1992a). Therefore, the interval from treatment with 5 mg E-17β to the emergence of the next follicular wave (approximately 4 days in norgestomet-treated heifers reported by Bó et al. (1995a) and in Section 6.0) was consistent with the time of resurgence of plasma FSH concentrations observed in ovariectomized cows (Section 5.0) and intact heifers (Bó et al., 1994). The interval from estradiol treatment to follicular wave

emergence was 4.3 days, regardless of day of treatment during the first follicular wave (Bó et al., 1995a). This is explainable by resurgence of FSH that followed estradiol-induced FSH suppression, depending on the circulating life-span of estradiol.

All estradiol formulations tested in this thesis suppressed plasma FSH concentrations, which remained low for varying periods, depending on the half-life of the estradiol form used. In Experiment 4 (Section 5.0), the administration of 5 mg E-17β, EB or estradiol valerate (EV) resulted in similar low circulating FSH concentrations by 12 to 36 h; however, FSH levels began to increase earlier and by 60 h were higher in E-17β- than in EB- or EV-treated ovariectomized cows (Section 5.0). Interestingly, the increase of plasma FSH concentrations in EB or EV groups was concomitant with relatively high concentrations of plasma estradiol (Section 5.0). Treatment with 5 mg EB induced a delayed and more variable day of follicular wave emergence than 5 mg E-17β or 1 mg EB (Section 6.0), or 2.5 mg EB (Caccia and Bó, 1998). These results supported the notion that high doses of the long-acting estradiol esters result in a delayed and more variable interval from treatment to follicular wave emergence in CIDR-B-treated cattle. This may be a consequence of a delay in the resurgence of FSH surge because of an extended suppression of FSH by EB or EV treatment.

By reducing the dose of long-acting estradiol esters, the interval from treatment to emergence of the new follicular wave can be made shorter and less variable. As mentioned above, a dose of 1 mg EB in ovariectomized cows resulted in a similar pattern of plasma FSH suppression to 5 mg E-17β and the interval to the emergence of the next follicular wave (3 to 5 days) was similar in intact heifers (Section 5.0, 6.0). This

pattern was also observed in ovariectomized heifers treated with 1 mg EB (O'Rourke et al., 2000). It has also been reported that the administration of 1 mg of EB on Day 13 of the estrous cycle in dairy cows resulted in synchronization of follicular growth, confirmed by the emergence of a third follicular wave (Burke et al., 1999). These findings were confirmed by our studies in CIDR-B-treated heifers; the injection of 1 mg EB resulted in synchronous emergence of a new follicular wave (Section 6.0). Based on the results after a reduced dose of EB in our studies, we speculate that the administration of reduced doses of EV may also result in a more precise interval to follicular wave emergence. The use of a reduced dose of EV has not been explored in intact animals Future studies to evaluate the effectiveness of reduced doses of EV on the manipulation of follicular development are necessary.

It was observed that when estradiol plus progesterone treatment was administered at the end of the luteal phase, it failed to cause atresia of the dominant follicle and synchronize follicular wave emergence. In CIDR-B-treated cattle, when follicular wave emergence occurred prior to treatment and spontaneous regression of the CL had already commenced, treatment with 5 mg E-17β or 1 mg EB was unsuccessful to synchronize follicular wave emergence (Section 6.0). In previous studies, E-17β given on Days 1, 3, or 6 after ovulation in norgestomet-treated intact heifers, suppressed follicular growth and the emergence of a new follicular wave was synchronized in 94% of heifers (Bó et al., 1995b), whereas in the remaining 6% of heifers, new wave emergence did not occur. Studies on the effect of estradiol or estradiol and progesterone given at the end of the estrous cycle are needed.

In earlier 12-day PGF-based estrus synchronization studies, EB was given 40 to 48 h after the second PGF injection, resulting in increased numbers of cattle in estrus, but pregnancy rates to AI were not increased (Peters et al., 1977). Treatment with EB also resulted in more heifers in estrus within 48 h after PGF treatment in a CIDR-B-based protocol (Hanlon et al., 1996). Treatment with E-17β in intact heifers with low plasma progesterone concentrations resulted in an LH surge by 18 to 24 h (Bó et al., 1994) and in ovariectomized cattle by 20 to 32 h (Hausler and Malven, 1976). Recently, it has been reported that treatment with EB 24 to 30 h after CIDR-B removal resulted in a synchronous LH surge at 20 h in cows (1 mg) and 16 h in heifers (0.38 or 0.75 mg; Lammoglia et al., 1998). In our studies, the length of the interval from estradiol treatment to the LH surge depended on when the treatment was given after progesterone removal. Estradiol benzoate administered at 12, 24 or 36 h after CIDR-B removal, induced an LH surge in all treated heifers (Section 6.0). When EB was given 12 h after CIDR-B removal the interval from treatment to the LH surge (~26 h) was longer than when EB was given at 36 h after CIDR-B removal (15 h; Section 6.0). The interval from estradiol injection at 24 h after CIDR-B removal to the LH surge was intermediate (20 h; Section 6.0). Although progesterone declined to concentrations less than 1 ng/mL 24 after CIDR-B removal, sampling was not frequent enough to detect when this occurred. Low concentrations of progesterone may have influenced the LH surge in heifers treated with EB at 12 h, whereas the effect of progesterone would have been minimal or nil in heifers treated with EB at 24 or 36 h. The LH surge in heifers treated with EB at 36 h may have been near the time of the spontaneous LH surge, which occurred 78 h after CIDR-B removal in Control heifers (Section 6.0). Control of LH secretion after CIDR-B

removal in an ovulation synchronization program involved an interaction between the exogenous and endogenous estradiol and progesterone. Estradiol benzoate given 24 h after PGF treatment (CIDR-B removal or 48 h after the last feeding of MGA) was used in our field experiments. High pregnancy rates to fixed-time AI 28 to 30 h after EB treatment (Section 7.0, 8.0, 9.0) suggested that this schedule may be appropriate for high pregnancy rates to fixed-time AI and practical from the point of view of cattle handling and management. Treatment at 12 or 36 h would compromise a morning/evening schedule of treatments, which would be more difficult to be carried out in field conditions by beef cattle producers.

The treatment with estradiol given after progestin removal resulted in almost 100% estrus rate. However, results of our experiments also suggested that treatment did not always result in ovulation at a predictable time (Section 6.0). In some heifers that had emergence of a follicular wave near the time of CIDR-B removal, EB treatment given 24 h after CIDR-B removal did not result in ovulation during the interval from 60 to 84 h after CIDR-B removal (those heifers ovulated between 84 and 108 h after CIDR-B withdrawal). However, it has been shown that treatment with 0.75 mg EB 500 Kg^{-1} BW given 24 h after CIDR-B removal induced ovulation of follicles that emerged 2 or 5 days before estradiol treatment; there was also no effect of follicle age on the interval to ovulation (Burke et al. 2001). In postpartum cows, follicles that arose one day prior to CIDR-B removal failed to ovulate in four of nine cows treated with EB 24 h after CIDR-B removal (Burke et al., 2001). The effect of maturity of the ovulatory follicle at the time of EB treatment remains controversial. Further studies should be conducted to

determine the effect of EB on the interval to ovulation in cattle at various intervals after follicular wave emergence.

The use of estradiol and progesterone to synchronize follicular wave emergence and estradiol to induce ovulation has been very effective in progestin-based synchronization protocols for fixed-time AI (Sections 6.0, 7.0, 6.0, 9.0). However, pregnancy rates to fixed-time AI in field experiments in which estradiol and progesterone were used to synchronize follicular wave emergence and estradiol was used to synchronize ovulation in PGF-based programs did not appear to be as high, especially compared to fertility in CIDR-B based protocols (Sections 7.0, 9.0). In the two-dose PGF protocol, EB administered 7 days after the first PGF treatment seemed to induce "early" estrus occurred in some heifers (Section 11.0), which was attributed to a luteolytic effect of the EB treatment given on Day 7. Estradiol has been previously used in estrus synchronization protocols to cause luteolysis (Wiltbank et al., 1961; Wiltbank and Kasson, 1968). In PRID-treated cattle, the administration of 5 mg EB reduced plasma progesterone concentrations from days 2 to 5 after treatment (Munro and Moore, 1985). However, small doses of EB (200 μg) did not affect the luteal life span (Hixon et al., 1983). Results of our studies suggest that 2 mg EB was marginally luteolytic. A single injection of progesterone given early in the estrous cycle has also been shown to lead to decreased CL weight, the proportion of functional luteal cells and progesterone production (Loy et al., 1960). Daily progesterone treatment starting three days after estrus has been also shown to shorten the length of the estrous cycle (Ginther, 1971). Because the first PGF injection in our protocol was given at random stages of the estrous cycle, great variability in follicular development was expected (Kastelic et al.,

1990a). Heifers may have ovulated from 1 to 6 days after treatment while others may have not ovulated at all. Therefore, when 2 mg EB and 50 mg progesterone were administered 7 days later, heifers were expected to be at different stages of luteal development, which may have resulted in a CL highly sensitive to estradiol treatment. There may not have been a single luteolytic effect of EB, but a synergistic luteolytic effect of EB and progesterone in heifers cannot be ruled out.

In a second study in which E-17β was used in lieu of EB, estrous behaviour was displayed only after the second PGF treatment, and there were no heifers detected in estrus before the second estradiol treatment. Since E-17β did not appear to have detrimental effects on the CL when given with progesterone, early estrus in some heifers in Experiment 1 (Section 11.0) was attributed to the luteolytic effect of 2 mg EB given on Day 7 after PGF treatment. Furthermore, as progesterone was given in both experiments, estradiol would appear to be the factor that induced luteolysis. Both estradiol formulations given with progesterone to synchronize follicular wave emergence followed by a single injection of 1 mg estradiol one day after the second PGF treatment resulted in acceptable pregnancy rates to AI after estrus detection. However, E-17β resulted in acceptable pregnancy rates to fixed-time AI, providing an alternative to progestin-based protocols for fixed-time AI in beef cattle.

In summary, administration of either E-17β or EB to synchronize follicular wave emergence, and a second treatment to induce ovulation, resulted in increased estrus rates and improved pregnancy rates in CIDR-B- or MGA-treated cattle (Sections 7.0, 8.0, and 9.0). Furthermore, use of either estradiol ester facilitated fixed-time AI in heifers assigned to prostaglandin-based programs (Section 11.0). However, use of EB may

result in uterine-induced luteolysis and, therefore, some estrus detection should be done when this ester is used in a PGF-based program.

12.2 GnRH or LH to synchronize follicular wave emergence and ovulation in synchronization protocols

Another method to synchronize follicular wave emergence during follicle development in cattle is to induce ovulation of the dominant follicle. GnRH and LH are the hormones of choice for ovulation induction (Macmillan and Thatcher, 1991; Twagiramungu et al., 1995; Martínez et al., 1999). Administration of exogenous GnRH stimulated LH and FSH release (Martínez et al., 2002) and the interval from treatment with GnRH or LH to the emergence of a new follicular wave has been reported to be approximately 2 days (Pursley et al., 1995; Twagiramungu et al., 1995; Martínez et al., 1999). However, ovulation occurred only when an active dominant follicle was present at the time of treatment (Kastelic and Mapletoft, 1998; Martínez et al., 1999). It was further shown that these hormones effectively synchronized follicular wave emergence only if ovulation of the dominant follicle occurred (Martínez et al., 1999). However, there was an additional number of animals in which a spontaneous emergence of new follicular wave occurred within 2 to 3 days after treatment (Martínez et al., 1999). This makes this approach feasible for estrus synchronization and fixed-time AI.

The use of GnRH for manipulation of follicular growth has been investigated in cattle (Macmillan and Thatcher, 1991; Drost and Thatcher, 1992; Thatcher et al., 2001). Synchronization of follicular wave emergence using GnRH has been also used in estrus

synchronization programs (Thatcher et al., 1993; Twagiramungu et al., 1995). In previous studies, a single dose of GnRH was incorporated into estrus synchronization programs to cause ovulation of the dominant follicle and emergence of a new follicular wave, followed 6 or 7 days later by administration of PGF to cause lysis of the induced and original CL (Twagiramungu et al., 1992; Thatcher et al., 1993). Protocols including two injections of GnRH given 9 days apart with PGF 2 days before the second treatment have been developed and named "Ovsynch" protocols (described in Section 1.0). These protocols have resulted in acceptable fertility to fixed-time AI performed at 0 to 24 h (optimum 16-18 h) after the second GnRH in dairy cows, but not in dairy heifers (Pursley et al., 1995; Wiltbank, 1997). Different modifications to this protocol have been proposed to increase efficacy such as the reduction of number of handlings (Cosynch protocol), different intervals between the first GnRH and PGF treatment, and different times of insemination (De Rensis and Peters, 1999).

In the Ovsynch protocol, a number of heifers have been detected in estrus prior to the scheduled fixed-time AI, especially during the interval from the first injection of GnRH to PGF treatment. This may be attributed to a low incidence of ovulation (~50%) to the first GnRH treatment (Purley et al., 1995; Martínez et al., 1999). In a previous report, there was no difference in estrus response or pregnancy rates to AI after estrus detection between groups of lactating dairy cows treated with an interval of 7 or 8 days between the first GnRH and PGF treatment (Thatcher et al., 1993). A shorter interval (6 days) was used in other studies, based on the notion that the number of females in estrus before the PGF treatment would also be decreased (Thatcher et al., 1989; Twagiramungu et al., 1992). A direct comparison of intervals showed that the precision

of estrus tended to be greater in heifers treated with a protocol consisting of an interval of 6 days over 7 days (Roy and Twagiramungu, 1999). Estrus rates during the intervals from GnRH to PGF treatment in lactating beef cows were similar (8.9 vs. 11.8 % for 6- and 7-day intervals, respectively) but fertility to fixed-time AI was not compared (Roy and Twagiramungu, 1999). When intervals of 6 and 7 days were compared in lactating beef cows in our studies, no differences in fertility were detected (Section 10.0). However, we did not compare 6- vs 7-day intervals in heifers in which failure of the first GnRH treatment and early estrus is more likely to occur. Further research should evaluate fertility after fixed-time AI following 6- and 7-day intervals from the first GnRH to PGF in heifers, as well as the use of a CIDR-B device in LH- or GnRH-based Ovsynch or Cosynch protocols with a 6-day interval in beef heifers.

Ovsynch protocols have not resulted in acceptable pregnancy rates in dairy (Pursley et al., 1995) or beef (Section 10.0) heifers. Administration of GnRH or LH treatment at different stages of development of the dominant follicle of the first follicular wave resulted in ovulation in 56% (GnRH) or 78% (pLH; Martínez et al., 1999) of heifers. Circulating progesterone concentrations at the time of treatment may have adversely affected the ovulatory response in GnRH-treated heifers; ovulatory response declined from Day 3 (89%), to Day 6 (56%) and to Day 9 (22%; Martínez et al., 1999). However, increasing concentrations of progesterone did not seem to affect response to the administration of pLH, since ovulatory response after pLH treatment was not different among days (67, 100 or 67% for heifers treated on Days 3, 6 or 9 after ovulation). Stage of the dominant follicle also affected the ovulatory response. Dominant follicles smaller that 8 mm in diameter did not ovulate after treatment, while

50% of dominant follicles of 8 mm ovulated. It is expected that ovulation following the second GnRH treatment will be higher if the dominant follicle has been selected and acquired LH receptors prior to treatment (reviewed in Section 1.0).

It was hypothesized that fertility to fixed-time AI could be improved in an Ovsynch-type protocol in beef cattle by the incorporation of a progestin treatment during the interval between the first GnRH injection and PGF treatment. This hypothesis was supported in heifers since the insertion of a CIDR-B device between the first GnRH or LH injection and PGF resulted in a two-fold improvement in pregnancy rates. However, no improvement was observed in lactating beef cows (Section 10.0). The increased fertility after fixed-time AI in heifers was attributed to suppression of estrus and ovulation during the GnRH-PGF interval. However, high plasma progesterone concentrations following the insertion of the CIDR-B devices may have reduced circulating LH concentrations for a period of 72 h, with the consequent atresia of an LH-dependent dominant follicle, followed by the emergence of a new follicular wave 2 to 3 days later (Cavalieri et al., 1998). In other words, the use of a CIDR-B device may have caused regression of the dominant follicle, resulting in resurgence of FSH and a new follicular wave.

It was proposed that two mechanisms may have been involved in the elimination of the dominant follicle and the subsequent emergence of follicular waves in heifers treated with GnRH at CIDR-B insertion. The first mechanism is the induction of ovulation of the dominant follicle present at the time of treatment (approximately 50% of heifers; Martínez et al., 1999, 2000). The second is the suppression of growth of the dominant follicle that did not ovulate after the first GnRH or LH treatment, because of

high levels of progesterone. The CIDR-B devices would contribute to increased plasma progesterone concentrations for at least 72 h, amplifying the suppressive effect of endogenous progesterone on circulating LH, and the consequence would be regression of these ovulatory follicles. Finally, in other cattle that did not ovulate, a new wave may have emerged within one or two days of treatment and this degree of synchrony may be adequate for fixed-time AI.

In our studies, low plasma LH concentrations were observed for approximately 72 h after insertion of new or once-used CIDR-B devices in ovariectomized cows (Section 5.0). The elevated concentrations of progesterone may have resulted in decreased LH pulsatility (Price and Webb, 1988), which may have led to regression of LH-dependent dominant follicles (Ginther et al., 1996b). A study involving monitoring follicular development and hormonal patterns in heifers and cows treated with CIDR-B devices and Ovsynch-type protocols would help explain the fertility in these programs and confirm the mechanisms of action of a CIDR-B device hypothesized herein.

The inclusion of a CIDR-B device in a Cosynch protocol has not always resulted in improved fertility in beef cattle. Although overall pregnancy rates in the groups with or without a CIDR-B device in our studies (~43%) were acceptable for an Ovsynch type protocol (Pursley et al., 1995; 26%, Schmitt et al., 1996a; 53%, Geary et al., 2001; 41 to 44%, Small et al., 2001), the beneficial effect of CIDR-B devices on fertility was only observed in beef heifers. It has been shown that the use of a CIDR-B device in Cosynch protocols applied at different herd locations resulted in increased overall pregnancy rates (58%) in beef cows, compared to Control cows treated only with Cosynch (48%; Lamb et al., 2001). However, the results were similar among different farms and there was no

location in which CIDR-B devices resulted in a significant improvement in pregnancy rates. It is noteworthy that the use of CIDR-B devices in a Cosynch program resulted in increased pregnancy rates in anestrus cows (Lamb et al., 2001). Therefore, reproductive status could affect results in cattle treated with CIDR-B devices.

Body condition can profoundly affect fertility in cows assigned to Ovsynch-type protocols. Cows assigned to the Ovsynch protocol, in Experiment 2 of Section 10.0, had a lower body condition (1.5 to 2.0 in a 5-point body condition-scoring scale) than desired at the beginning of an AI program (2.5 to 3.5; Section 10.0). CIDR-B devices may not have a substantial suppressive effect on plasma LH concentrations, as cows under poor nutrition have with low LH concentrations (Murphy et al., 1991). In Section 8.0, beef heifers were gaining weight prior to treatment but they were transferred to a diet of only roughage, and pregnancy rates to fixed-time AI were disappointingly low. An increase of one unit in a 0 to 9 body condition-scoring scale resulted in an increase of 16.3% in the proportion of cyclic cows (Lamb et al., 2001). It appeared that CIDR-B devices resulted in improved fertility in a Cosynch program when applied to cattle with an optimum body condition. An important consideration to take into account before applying these protocols for fixed-time AI in a beef herd is the careful observation of body condition of females.

Similar results were obtained after the use of either pLH or GnRH in Ovsynch-type protocols. Ovulation rate after a single LH treatment was higher than after GnRH treatment in beef heifers (78 vs 56%, respectively; Martínez et al., 1999). Treatment of beef heifers with 25 mg pLH on Day 6 of the first follicular wave resulted in 100% ovulation (Martínez et al., 1999) and response depended only on the stage of

development of the dominant follicle. However, cattle under field conditions are almost invariably asynchronous and therefore a uniform ovulatory response in the herd to LH treatment has not been observed (Oswald et al., 2000). Although not directly compared, fertility after the administration of 12.5 mg LH in Ovsynch-type protocols (with or without CIDR-B devices) was almost identical to that in protocols using a dose of 100 μg GnRH (Section 10.0). Porcine LH has been used to induce ovulation in pregnant animals (Lulai et al., 1994) or to induce ovulation of superstimulated follicles in embryo transfer programs (D'Occhio et al., 1999), but there has been no previous research regarding the use of pLH in ovulation synchronization programs for fixed-time AI. This gonadotrophin has been shown to be an efficacious alternative to GnRH in Ovsynch-type synchronization protocols, especially in beef heifers, when a CIDR-B device has been inserted between the first treatment and PGF. In addition, the dose of LH has been reduced to less than one-half recommended dose, resulting in an acceptable ovulatory response at the beginning and end of an LH-based Ovsynch program (Oswald et al., 2000). Nevertheless, experiments designed to critically determine the minimum dose of LH at different stages of the estrous cycle and dominant follicle development are necessary before use of pLH could be recommended as a replacement for GnRH.

12.3 Practical aspects of the use of estradiol/progesterone-estradiol, GnRH or LH in synchronization protocols.

In the induction of estrus and ovulation, estradiol is the key hormone produced by the preovulatory follicle that commands all signs associated with estrus, LH release

and, in turn, ovulation. Estradiol administered at an appropriate dose has resulted in a high degree of synchrony in emergence of an ovarian follicular wave (Bó et al., 1995a; Burke et al., 1997a, 1997b; Section 6.0). The combination of estradiol and progesterone has been shown to affect different stages of follicular growth at the time of treatment (i.e., dominant follicle before and after selection), while the emergence of the new follicular wave depended on the life-span of estradiol in the circulation. On the other hand, GnRH has been partly effective in inducing ovulation of the dominant follicle (Pursley et al., 1995), and the emergence of a new follicular wave has depended on the elimination of that follicle (Pursley et al., 1995, Martínez, 1999, 2000). Ovulation is likely to occur after the acquisition of LH receptors which occurrs at the time of selection of the dominant follicle (i.e., follicles greater than 8 mm in diameter; Xu et al., 1995). However, ovulation of a selected dominant follicle does not always occur following GnRH treatment; some follicles greater than 8 mm in diameter did not ovulate in response to GnRH treatment (Martínez et al., 1999).

The treatment with estradiol resulted in a greater number of heifers detected in estrus than with GnRH or LH (Sections 7.0, 9.0). Estradiol was more consistent than GnRH or LH in inducing estrus in either CIDR-B- or MGA-based synchronization protocols (Section 9.0). Estrus rates by 60 h after PGF injection in estradiol-treated groups ranged from 92 to 100%, whereas estrus rate in GnRH or LH-based Ovsynch-type protocols ranged from 20 to 40% (Sections 7.0, and 10.0). When the second GnRH treatment was delayed until 72 h, estrus rates increased to 56.7% (Section 8.0). Low estrus rates (20%) have reported before the time of the second GnRH injection at 48 h after PGF treatment in cows and some of those (5%) were even detected in estrus before

PGF (DeJarnette et al., 2001). Others have reported a relatively high proportion of cows detected in estrus before PGF in Ovsynch protocols (11%; Mialot et al., 1999) and heifers (17%; Roy and Twagiramungu, 1996). These animals would have not conceived to fixed-time AI after the second GnRH injection and any approach to the improvement of Ovsynch fixed-time AI programs must address this issue.

From a practical point of view, estradiol treatment ensured a vaginal discharge of estrous mucus and more fluid in the cervix at 'induced' estrus, which facilitated AI, especially when a high number of females were inseminated. Even though vaginal mucus and ease of cervical passage by AI technicians have been reported as not being predictors of pregnancy (Loeffler et al., 1999), they are indicative that a female is in estrus and it will affect the manner that insemination is performed. The more fluid in the cervix, the easier cervical passage and less roughness associated with the procedure. Fixed-time AI of heifers detected in estrus resulted in high fertility (Section 9.0) and those observed in estrus had higher pregnancy rates than those not detected in estrus, regardless of the treatment group. This also confirmed the high fertility observed after AI in spontaneous estrus in dairy herds (Sturman et al., 2000).

Estradiol has been shown to facilitate sperm transport in the female reproductive tract (Harper, 1994). It has been reported that treatment with 10 mg of EB increased the sperm count in oviducts, uterus and cervix after mating (Hawk and Cooper, 1975). In contrast, GnRH or LH may induce ovulation of follicles that may have not reached maturity, as evidenced by low estradiol influence at AI with "dry" cervix, reported by the inseminator (Section 7.0). This could result in impaired sperm transport. Although estrus rates were low (suggesting low estradiol concentrations), treatment with GnRH

did not appear to compromise the follicular environment and its capacity to yield a competent oocyte (Twagiramungu et al., 1995). In our studies, highly acceptable pregnancy rates ranging from 50 to 68% were achieved after Cosynch and Ovsynch protocols in which GnRH or LH was used in CIDR-B-treated heifers (Sections 7.0, 9.0, 10.0). It has been reported that follicles of 8 or 9 mm (present at the time of treatment) will ovulate following a single GnRH treatment (Martínez et al., 1999). Ovulation of small follicles may result in decreased plasma estradiol concentrations, small CL size, low plasma progesterone concentrations, and reduced fertility (Vasconcelos et al., 2001). Although the use of estradiol to synchronize estrus and ovulation appeared to be more physiologic than the use of GnRH or LH, there is no assurance that the follicle that ovulated was more mature or that the CL formed was more functional. The use of one type of treatment over the other did not affect pregnancy rates at fixed-time AI (Section 9.0).

Regarding movement of cattle to administer treatments, estradiol-based protocols require one more cattle handling than GnRH- or LH-based Cosynch programs, due to the treatment given to induce ovulation. However, estradiol treatments have resulted in similar pregnancy rates demonstrating that the stress related to one more handling did not aversely affect results. The use of other estradiol esters such as estradiol cypionate may be an option for fewer passages of cattle through the chute. In one study, estradiol cypionate administered at the time of CIDR-B removal and PGF injection has resulted in acceptable pregnancy rates to fixed-time AI (Colazo et al., 2002). However, fertility was not as high as when estradiol cypionate was given at 24 h after CIDR-B removal and PGF treatment (Colazo et al., 2002). Numbers of handling

appeared to have no great influence in pregnancy rates. When estradiol-, GnRH- or pLH-based protocols (with four or three trips through the chute, respectively) were directly compared, pregnancy rates following fixed-time AI were similar among groups (Section 9.0).

12.4 Progestins and endogenous progesterone in synchronization protocols.

Results of several experiments in this thesis suggested that the oral progestin (MGA) and the intravaginal device (CIDR-B) are equally efficacious; however, CIDR-B has resulted in a higher number of females in estrus and in numerically higher pregnancy rates than MGA treatment. Even in the same experiment (Section 9.0), there was a tendency for higher pregnancy rates in CIDR-B-treated groups than MGA-treated groups, especially when these progestin treatments were used in combination with Ovsynch-type programs. It has been reported that exogenous progestins are not given at a dose sufficient to result in the same effects on gonadotrophins (LH) as with endogenous progesterone from the CL (Kojima et al., 1995). Therefore, at the common used dose, progestational treatments suppress estrus and LH peak, but are not physiological.

As discussed in Section 1.0, traditional long-term MGA programs have resulted in low fertility (Odde, 1990). In our studies, a low pregnancy rate (~30%) was obtained with a short-term MGA program in which follicular wave emergence was not synchronized (Control group of Experiment 1, Section 6.0), compared with estradiol- or GnRH-treated groups. The reason for low fertility in regimes without synchronization of

follicular wave emergence is that either progestin could induce a persistent follicle if luteal regression was occurring during the time of treatment (Custer et al., 1994; Patterson et al., 1989). Oocytes from persistent follicles apparently undergo in vivo maturation (Revah and Butler, 1996), which is initiated when a period of dominance exceeds 8 days before the LH surge (Mihm et al., 1999). However, if the emergence of follicular waves was synchronized within the treatment period, the development of a new dominant follicle capable of ovulating a healthy oocyte would overcome this drawback in MGA programs (Kastelic et al., 1996), and this newly recruited dominant follicle can be induced to ovulate in fixed-time AI programs, with acceptable fertility (Sections 6.0, 9.0 and 10.0).

The treatment groups for synchronization of follicular wave emergence in experiments in Sections 7.0 and 10.0 were identical, even though MGA replaced a CIDR-B device during the period between the first treatment with EB, GnRH or pLH and PGF. For example, in Section 7.0, pregnancy rates after fixed-time AI in an estradiol/CIDR-B-based program averaged 76 and 63% in heifers and cows, respectively; while pregnancy rates were 55.7% in cows and heifers when the same protocol was applied using MGA during the first 6 days after the beginning of the experiment. Another comparison was performed in Section 9.0, in which these protocols were studied in a single experiment. Although there was no significant difference (most likely due to number of heifers in each group), overall pregnancy rates in MGA-treated (56%) heifers were numerically lower than in CIDR-B-treated (61%) heifers, due to the pregnancy rates in groups treated with GnRH (52 and 65% in MGA and CIDR-B groups, respectively). Inconsistent consumption of MGA may have played an important

role in those groups (Fike et al., 1999). However, problems with MGA intake would be more likely to occur in cattle on pasture than in cattle fed in dry lot conditions (Fike et al., 1999). It has been also observed that MGA does not mimic endogenous progesterone regulation (Kojima et al., 1992). Mean plasma LH concentrations and frequency of LH pulses were greater in cows treated with MGA when the CL was not present (Kojima et al., 1995). A high dose of MGA (three times the recommended dose) resulted in less LH suppression than progesterone produced when the CL was present (Kojima et al., 1995). In our field studies, it was not possible to collect information on circulating gonadotrophins. However, there was no evidence of an effect of LH suppression by MGA, as occurred with CIDR-B devices during the first 48 to 72 h after insertion (Section 5.0) that would cause regression of the dominant follicle. Studies that monitor ovarian follicle and luteal dynamics in relation to the use of MGA and treatments to synchronize follicular wave emergence and ovulation should be designed. In addition, hormonal and ultrasonographic evaluation of Ovsynch-type programs in MGA-treated cattle should continue.

13.0 GENERAL CONCLUSIONS

The hypothesis that the synchronization of ovarian follicular wave emergence and ovulation will facilitate fixed-time artificial insemination and result in pregnancy rates comparable to insemination after detected estrus was supported by the results obtained in this thesis. New synchronization protocols that facilitated fixed-time artificial insemination were developed and fertility was highly acceptable. In addition, basic information on the effects of steroid or protein hormones on follicular development was generated, providing the basis by which treatments could be improved. The following are the general conclusions based on results of the experiments in this thesis and the current information that has been discussed:

1 - The synchronization of follicular wave emergence and ovulation in an estrus synchronization protocol for cattle facilitated fixed-time AI;

2 - The administration of either estradiol-17β or estradiol benzoate in a 7-day progestin-based protocol in beef cattle synchronized follicular wave emergence and ovulation, which facilitated fixed-time AI with high fertility;

3 - The emergence of a follicular wave depended on the resurgence of plasma FSH concentrations following estradiol-induced FSH suppression or GnRH/pLH induced ovulation;

4 - The duration of FSH suppression depended on the circulating life-span of the estradiol and the dose used;

5 - In a two-dose prostaglandin synchronization protocol, the administration of either E-17β or EB for the synchronization of follicular wave emergence and ovulation facilitated fixed-time AI in beef heifers with acceptable fertility. However, EB caused uterine-induced luteolysis in some heifers;

6 - Both GnRH and pLH were succesfully used in Ovsynch-type programs in cows;

7 - The duration of the interval from the first GnRH to PGF treatment in an Ovsynch-type protocol did not affect pregnancy rates in lactating beef cows;

8 - The insertion of a CIDR-B device during the interval from the first GnRH/pLH to prostaglandin in Ovsynch-type synchronization protocols significantly improved pregnancy rates to fixed-time AI in beef heifers;

9 - Progesterone from CIDR-B devices suppressed plasma LH release and induced regression of the dominant follicle;

10 - Progesterone given either intravaginally or intramuscularly was not capable of blocking the estradiol-induced LH surge in ovariectomized cows.

14.0 SUGGESTIONS FOR FUTURE RESEARCH

The studies reported in this thesis answered the questions related to our main hypothesis (Section 3.0). However, these studies also provided for the formulation of new hypotheses. The experiments that examined dose and forms of estradiol are the basis for new studies to fine-tune of synchronization of follicular wave emergence, follicular growth and ovulation. Responses following treatment with estradiol and progesterone should be carefully investigated to assess the optimum proportion of each steroid in controlling the development of FSH-dependent or LH-dependent follicles. Resurgence of FSH following estradiol-induced FSH suppression depended on the circulating life-span of estradiol. Research on dose and the use of other estradiol esters could lead to more successful alternatives for the manipulation of the ovarian function. In that regard, studies to evaluate the effectiveness of reduced doses of EV on follicular development are needed. Studies of specific doses of EB, with or without progesterone, in two-dose prostaglandin synchronization protocols should also be initiated. Finally, synchronous emergence of follicular waves facilitated synchronization of ovulation and high fertility to fixed-time AI. However, research should focus on the optimal interval from the emergence of a dominant follicle to ovulation that will result in the highest fertility.

The simultaneous comparison of treatments that affect gonadotrophins in intact and ovariectomized cattle would provide insight into hormone dynamics. The same treatment protocols could influence to each category of cattle differently. In addition, the

stage of the estrous cycle may also be important, since it was observed that treatment with estradiol and progesterone after luteolysis had been spontaneously induced, did not result in follicle suppression and follicular wave emergence. The study of the effect of estradiol with or without progesterone given at the end of the estrous cycle would identify the cause of possible failure of such a treatment.

Studies that included a CIDR-B device in an Ovsynch protocol reported herein provide the basis for more detailed research on ovarian physiology in different categories of cattle. This would result in a better understanding of the use of CIDR-B in Ovsynch programs in heifers, and of the potential effect of CIDR-B devices or MGA in Ovsynch protocols in cows. Although results of induction of ovulation and fertility indicate that GnRH or pLH have similar effects on cattle, a direct comparison of these hormones in an Ovsynch program with monitoring of follicular dynamics and fertility to fixed-time AI would also be valuable. Experiments to determine the minimum dose of pLH at different stages of the estrous cycle and dominant follicle development should be initiated before widespread use of pLH in synchronization programs is recommended.

Ultrasonographic and hormonal monitoring of ovarian follicles and luteal dynamics during and after MGA feeding (with or without treatments to synchronize follicular wave emergence and ovulation) would increase the understanding of follicular dynamics in MGA-based protocols.

Additional studies must address the precision of ovulation required to achieve optimal timing for high fertility to fixed-time AI. The effect of maturity of the ovulatory follicle at the time of estradiol-induced LH release remains controversial. Further studies

should be conducted to clarify the effect of EB on the interval to ovulation in heifers and cows at various intervals after follicular wave emergence. Furthermore, methods of synchronization of ovulation that require less cattle handling and management should be considered to increase producers' confidence in AI programs.

Body condition is also an important factor that strongly affects fertility when applying synchronization protocols for fixed-time AI in a beef herd. Nutritional management cannot be replaced by a hormonal treatment. The effect of body condition and feeding regimens on follicular development and response to hormone treatment must be investigated. Complementary studies on feeding prior to and during synchronization protocols will contribute to increase fertility in protocols following fixed-time AI. In conclusion, the design of a synchronization protocol that requires minimal cattle handling, low cost and results in high fertilitymust be the ultimate goal of studies involving fixed-time artificial insemination.

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